IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :

U.S. National Serial No. :

Filed :

PCT International Application No. : PCT/FR2003/003336

VERIFICATION OF A TRANSLATION

I, Charles Edward SITCH BA,
Deputy Managing Director of RWS Group Ltd UK Translation Division, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare:

That the translator responsible for the attached translation is knowledgeable in the French language in which the below identified international application was filed, and that, to the best of RWS Group Ltd knowledge and belief, the English translation of the international application No. PCT/FR2003/003336 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: April 12, 2005

Signature :

For and on behalf of RWS Group Ltd

Post Office Address : Europa House, Marsham Way,
Gerrards Cross, Buckinghamshire,
England.

NOVEL AMPHIPHILIC FLUOROCARBON MOLECULAR VECTORS FOR BIOMEDICAL AND MEDICAL USE

The invention relates to novel molecules that can be used as vectors for active principles, to active molecules comprising such a vector and to the use thereof in the pharmaceutical field, in particular for preparing medicinal products.

Current research on the delivery of active principles tends to notably improve not only the patient's comfort by favoring the least traumatic routes of administration, but also the overall effectiveness of a medicinal product by conferring on it a cellular affinity that it generally lacks and that is often responsible for adverse side effects. Added to the conventional concept of therapeutic activity is therefore that of the specificity of action, which in fact modulates the bioavailability of the substrate.

In practice, for example in a sensitive field such as that of anticancer chemotherapy, the progress observed over the last few years (novel drugs, novel methods of administration, chemopredictivity *in vitro* or *in vivo*, integration into novel therapeutic schemes, etc.) makes it possible to think that the quality of chemotherapy will continue to progress if, from now on, it can be equipped with novel tools based on novel concepts for transport and cellular targeting of the anticancer agents. In order to increase the effectiveness and the effective dose of active principle which will be conveyed to the cancerous site, it in fact appears to be necessary to give these medicinal products a real specificity and to reduce their side effects.

Now, the chemical structure of a medicinal product conditions both its physicochemical properties and its biological activity, and in particular its affinity for

membrane receptors. Modulating this outcome through modifications of the molecular structure risks altering the pharmaceutical properties. One is therefore led to treat the product from a "galenic" point of view, i.e. to take an interest in the pharmaceutical form used for its administration, or better still to encapsulate it in host structures or graft it onto molecules capable of providing this vectorization.

The vectorization of active principles must take into account several parameters in order to have a chance of success:

As has just been specified, the active principle should, as much as is possible, be isolated from the physiological medium in order to prevent any interaction which is harmful to the medicinal product or to the organism.

The carrier should not impair, or better still should improve, the bioavailability of the medicinal product. In other words, the therapeutic agent should be released within the target molecule (preferably at the intracytoplasmic level, in certain cases at the intranuclear level) and should conserve therein its entire activity.

However, this vision of the medicinal product, an agent specific for a receptor, that is capable of causing a cellular response and, as a result, of correcting a deficiency is far from being general. While it is correct for hormone or analgesic-type active principles, it does not apply at all to other fields, for instance anticancer treatments. Such substrates were not designed in this way: they do not have any cell recognition specificity. Their aim is to inhibit cell multiplication. They are generally antimitotic agents and, as a result of this, they can act on the DNA of all cells, whether they are cancerous or normal

and can thus create certain bothersome conditions, the most common manifestation of which is hematopoietic tissue aplasia, to which is subsequently added immuno-inhibition and digestive problems. Since the establishment of chemotherapy, the active principles developed have been increasingly powerful, but unfortunately do not distinguish between cancer cells and normal cells. It is thus regrettable that effectiveness and selectivity in terms of cancer treatment cannot be conjugated on the same active principle.

Given these various considerations and observations, various vector models have been proposed, essentially of macromolecular type (synthetic or natural polymers) and supramolecular type (liposomes). Among all these vector models, mention may in particular be made of the development of small amphiphilic polymers, called telomers, capable of modulating the hydrophilicity-lipophilicity balance of the active principle (and therefore its intrinsic physicochemical properties), but also of promoting its intracellular penetration and of providing it with cellular targeting by means of suitably chosen recognition agents.

"Synthesis of new cotelomers derived from tris(hydroxymethyl)aminomethane bearing arabinofuranosylcytosine moieties. Preliminary results on their in vitro and in vivo antitumoral activities" C. Contino, J.C. Maurizis, M. Ollier, M. Rapp, J.M. Lacombe, B. Pucci. *Eur. J. Med. Chem.*, (1998), **33**, 809-816.

"Synthesis and preliminary biological assessments of a new class of amphiphilic telomers bearing 5-fluorouracil moieties" C. Contino, J.C. Maurizis and B. Pucci. *Macromol. Chem.*, (1999), **200**, 1351-1355.

"A new strategy in biomedical and medical field: the

synthesis and applications of telomeric structures". P. Barthelemy, A. Polidori, B. Pucci. *Transworld Research Network, Recent developments in organic chemistry, Trivandrum,* (1999), **3**, 117-140.

"Synthesis and Preliminary biological assessments of RGD bearing biocompatible telomers. Sylvain Jasseron, Christiane Contino-Pépin, Jean Claude Maurizis, Maryse Rapp, Bernard Pucci. *Bio. Med. Chem. Letters,* (2002), **12**, 1067-1070.

"Synthesis and preliminary biological assessments of a new class of amphiphilic telomers bearing 5-fluorouracil moieties" C. Contino, J.C. Maurizis and B. Pucci. *Macromol. Chem.*, (1999), **200**, 1351-1355.

"Amphiphilic telomers: a new kind of antimitotic drugs macromolecular carriers." Christiane Contino-Pépin, Jean-Claude Maurizis, Bernard Pucci. *Curr. Med. Chem.-Anti-Cancer Agents,* (2002), **2**, 645-665.

The results acquired in the course of these studies have made it possible to demonstrate various major points:

Control of the hydrophilicity-lipophilicity balance of the substrate promotes its transmembrane passage ("Uptake and subcellular distribution of a new fluorinated telomeric carrier: study on cultivated B16 melanoma and skin rat fibroblastic cells". F. Chehade, J.C. Maurizis, B. Pucci, A.A. Pavia, M. Ollier, A. Veyre, F. Escaig, C. Jeanguillaume, R. Dennebouy, G. Slodzian, E. Hindie, *Cellular and Molecular Biology,* (1996), **42**, 335-342) without, however, introducing a detergent and therefore toxic nature ("Efficiency of new non ionic telomeric surfactants towards the solubilization of subcellular fraction proteins" B. Pucci, J.C. Maurizis and A.A. Pavia, *BioOrg. Med. Chem Lett.* (1993), **3**, 161-164).

These amphiphilic polymers make it possible to provide the overall molecule and therefore the active principle with effective cell targeting ("Cell targeting by glycosidic telomers - Recognition ability of galactosylated telomers by the yeast *Kluyveromyces Bulgaricus*" J.Coulon, R. Bonaly, B. Pucci, A. Polidori, P. Barthelemy, C. Contino, *Bioconjugate Chem.* (1998), **9**, 152-159. "Permeability of yeast cell enveloppe to fluorescent galactosylated telomers derived from THAM". C. Contino, M. Briot, J. Coulon, A. Polidori, R. Bonaly and B. Pucci. *Bioconjugate Chem.*, (2000), **11**, 461-468. "Synthesis and Preliminary biological assessments of RGD bearing biocompatible telomers". Sylvain Jasseron, Christiane Contino-Pépin, Jean-Claude Maurizis, Maryse Rapp, Bernard Pucci. *Bio. Med. Chem. Letters*, (2002), **12**, 1067-1070).

Active principles grafted onto the vector by means of a suitable peptide spacer arm (that can be hydrolyzed by cytoplasmic enzymes) are released at the intracellular level after the vector has passed through the cell membrane ("Synthesis and Preliminary biological assessments of RGD bearing biocompatible telomers". Sylvain Jasseron, Christiane Contino-Pépin, Jean Claude Maurizis, Maryse Rapp, Bernard Pucci. *Bio. Med. Chem. Letters*, **12**, 1067-1070).

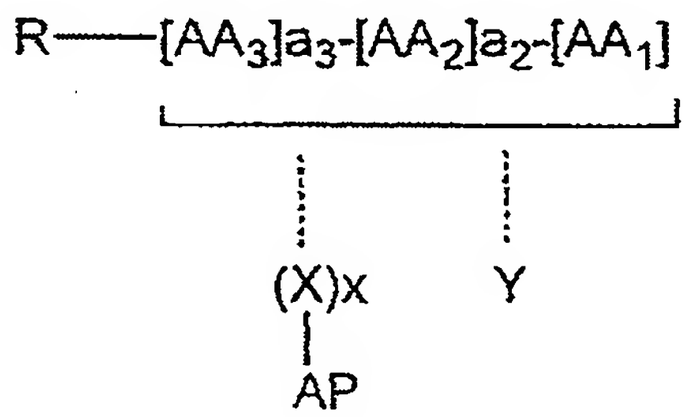
This method of vectorization makes it possible to very significantly increase the effectiveness of the anticancer agent since it inhibits the proliferation of metastases, slows down tumor growth, and prolongs the lifetime of treated mice by a factor of more than 3 compared with control mice.

Despite the obvious advantage provided by these telomers which have been described in document WO 92/02560, one of the major problems with which such vectors are confronted and which could disrupt the commercialization and the use thereof is their

polydispersity, i.e. their lack of well-defined mass and structure.

Thus, the Applicant set itself the objective of designing and preparing molecules capable of being vectors, for an active principle, which have a well-defined structure, the preparation of which is easy, and which facilitate the delivery of the active principle to its target.

A subject of the invention is therefore the molecules corresponding to formula (I) below:



(I)

in which:

AP represents the active principle capable of acting on a biological target and the delivery of which to its biological target it is desired to promote;

x represents an integer chosen from 0 and 1;

X represents a peptide chain comprising from 1 to 5 amino acids;

AA_1, AA_2 and AA_3, which may be identical or different, each represent an amino acid;

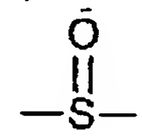
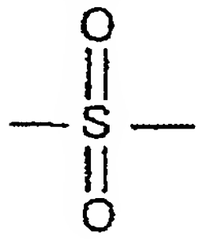
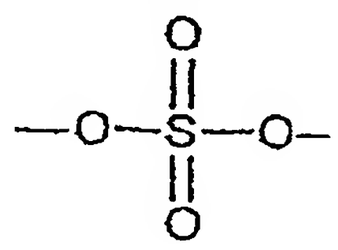
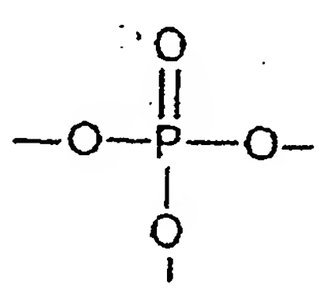
a_2 and a_3, which may be identical or different, each represent an integer chosen from 0 and 1;

R represents a group chosen from a targeting agent and a solubilizing agent. For the purpose of the present invention, the term "targeting agent" is intended to mean: a molecule that promotes the delivery of the entire molecule of formula (I) to its target or any molecule capable of being recognized by the target of the active principle AP. The term "solubilizing

agent" is intended to mean an agent for modulating the HLB balance of the molecule of formula (I), in particular a hydrophilic agent. Among the targeting agents that can be used in the present invention, mention may be made of monosaccharides, aminated derivatives of sugars, polysaccharides, natural or synthetic hormones, peptides, antibodies and, generally, any molecule capable of being recognized by the target of the active principle AP. Among the solubilizing agents that can be used in the present invention, mention may in particular be made of polyols, polyethers, peptides and polysaccharides.

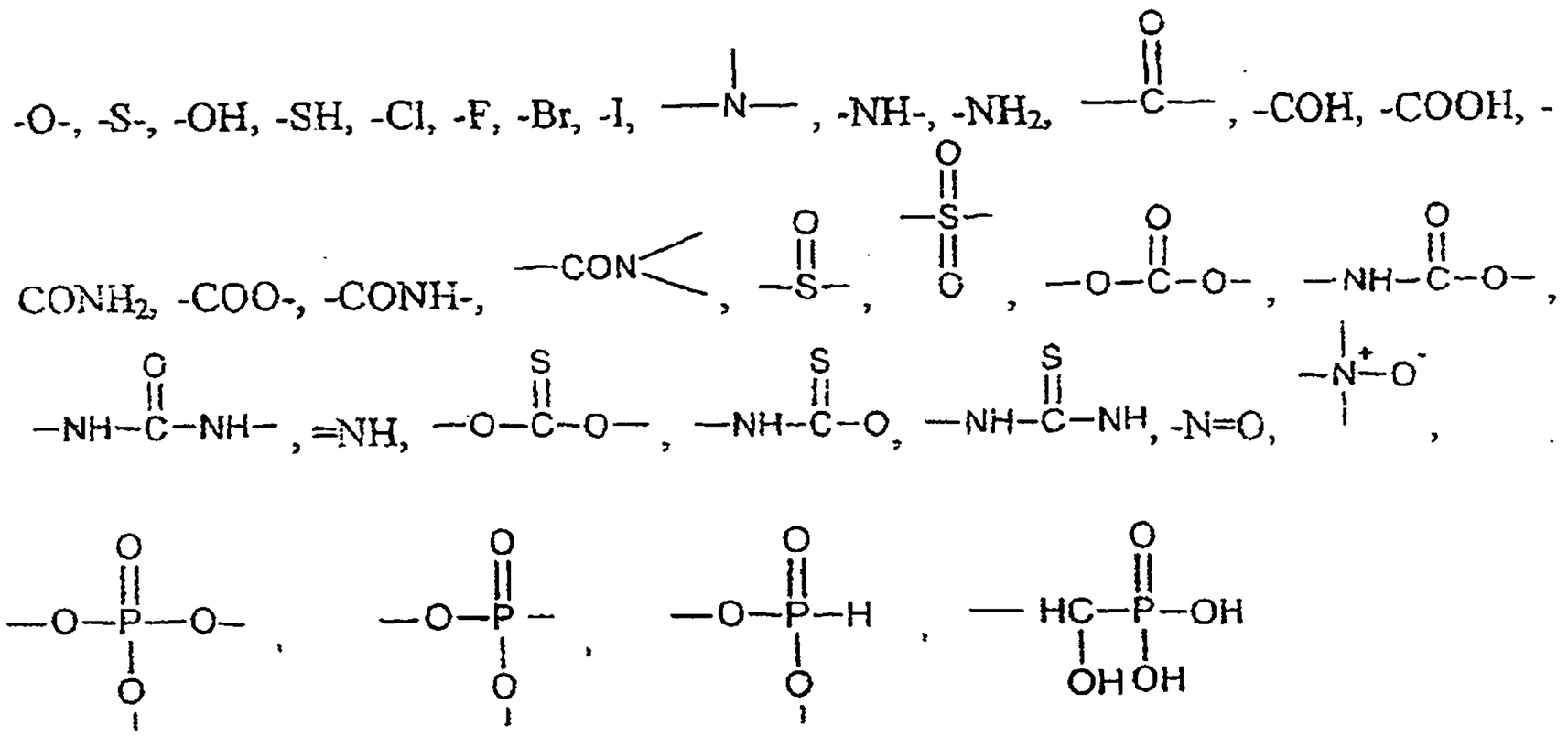
Y represents a fluorinated C_4-C_{12} hydrocarbon-based chain containing a group  , -NH-, -O-CO-NH-, S or O that allows its attachment, indicated by the dashes ---, either to one of the ends of the peptide chain $[AA_3]_{a3}$-$[AA_2]_{a2}$-$[AA_1]$, or to the side chain of one of the amino acids AA_1, AA_2 or AA_3;

The dashes --- between AP-$(X)_x$ and the chain $[AA_3]_{a3}$-$[AA_2]_{a2}$-$[AA_1]$ indicate that the linkage of AP-$(X)_x$ with the rest of the molecule occurs via the side chain of one of the amino acids AA_1, AA_2 or AA_3 or, optionally, at the end of the peptide chain.

More particularly, the active principle is chosen from all organic molecules that have a recognized biological activity and that are capable of being attached to an amino acid by means of a linkage that can be chosen from the functions -O-CO-, -CO-NH-, -NH-CO-NH-, -NH-CO-O-, O-CO-O-, -O-, -S-,  ,  ,  , 

Among these active principles, mention may in particular be made of those that have anticancer, anti-inflammatory, antiseptic, analgesic, neuroleptic or antifungal activity, and molecules that have free-radical scavenger activity.

In general, the active principle may consist of a linear, branched or cyclic molecule containing from 1 to 30 carbon atoms, one or more unsaturations, in particular one or more aromatic rings, and one or more functions chosen from:



The active principle AP is attached, by means of a linkage the nature of which was disclosed above, either to the side chain of one of the amino acids AA_1, AA_2 or AA_3, or to the end of the peptide chain, optionally by means of a peptide chain X (in the case where x=1).

The linkage with one of the two groups Y and -(X)x-AP takes place on the side chain of one of the amino acids AA_1, AA_2 or AA_3. The amino acid attached to AP-$(X)_x$- or to Y via its side chain is chosen from those containing an acid, amide, amine, thiol or alcohol function on their side chain. Among these, mention may in particular be made of lysine, arginine, ornithine,

aspartic acid, glutamic acid, asparagine, glutamine, serine, tyrosine or cysteine. Preferably, the amino acid attached via its side chain to AP-$(X)_x$- or to Y is chosen from: aspartic acid or lysine.

The spacer arm X, when it is present, consists of a peptide chain involved, at one end, in a linkage with the side chain or the end of one of the amino acids AA_1, AA_2 or AA_3 and, at the other end, in a linkage with the active principle AP.

This spacer arm comprises 1 to 5 amino acids, preferably 1 to 3 amino acids.

The spacer arm X and/or the peptide chain $[AA_3]_{a3}$-$[AA_2]_{a2}$-$[AA_1]$ can be chosen for their affinity for the target of the active principle AP. They may also comprise or consist of tyrosine residues that make it possible to follow the molecule of formula (I) *in vivo*, after labeling with ^{125}I.

R is chosen according to the cell target; it may be saccharide in nature (targeting of specific membrane lectins that are in specific tissues and that selectively recognize either galactose -in the case of the liver, of bones, of certain cancerous tumors-, or mannose -in the case of macrophages, of the heart-, or sialic acid -in the case of erythrocytes-, etc.), hormonal in nature (such as steroids), or synthetic in nature such as imatinib mesylate (ST571, Gleevek®) for targeting kinases, specific antibodies, in particular peptides. R can be chosen from any substrates for which prior research has demonstrated the recognition specificity. When R is a monosaccharide or polysaccharide or a hydrophilic peptide, it may, in addition, provide the molecule with the water-solubility necessary for its IV or IP administration.

When R is a peptide chain, R advantageously contains

from 3 to 15 amino acids, even more advantageously from 3 to 10 amino acids. It may also contain one or more tyrosine residues that make it possible to follow the molecule of formula (I) *in vivo*, after labeling with ^{125}I.

The amino acids constituting the spacer arm X, just like those constituting the chain $[AA_3]_{a3}$-$[AA_2]_{a2}$-$[AA_1]$ or the group R are chosen from natural amino acids such as alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine, or non-natural amino acids such as hydroxyl-proline, norleucine, ornithine, citruline or cyclohexylalanine.

The use of Ω-amino acids such as 3-aminopropionic acid and 4-aminobutyric acid can also be envisioned.

When R is a peptide, the peptide chain R may be an antibody fragment or epitope having a pronounced affinity for the AP's biological target.

Among the peptides that can be used in the present invention, mention may be made, for example, of: the RGD sequence, known for its affinity for αVβ3 integrins.

R can also be chosen from polyols or polyethers, in particular poly(ethylene oxide)s, so as to give the molecule of formula (I) a hydrophilic/lipophilic balance that promotes its solubility in water and its penetration into the cell as far as the target of the active principle AP.

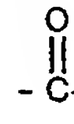
When R consists of a polyol, said polyol advantageously consists of an alkyl chain comprising from 4 to 16 carbon atoms and from 4 to 16 hydroxyl groups.

When R consists of a poly(ethylene oxide) chain as solubilization unit, said chain advantageously comprises from 5 to 30 ethylene oxide units.

R can in particular be chosen from monosaccharides, aminated derivatives of sugars, and polysaccharides.

Among the monosaccharides that can be used in the present invention, mention may be made of: glucose, fructose, mannose, galactose and ribose. Among the aminated derivatives of sugars, mention may in particular be made of glucosamine. Among the polysaccharides that can be used in the present invention, mention may be made of lactose, cellobiose or maltose, lactobionamide and sucrose. Preferably, the polysaccharide chains used in the invention are bisaccharides.

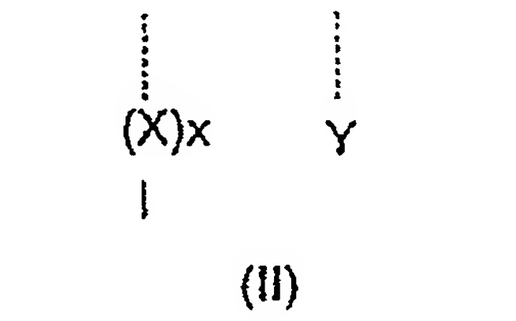
The attachment of R to one of the ends of the chain $[AA_3]_{a3}-[AA_2]_{a2}-[AA_1]$ takes place by means of a suitable linkage: ether, amide, carbamate, thioether, ester, urea, urethane, according to the functionality that can be grafted onto R.

The fluorinated hydrocarbon-based chain is preferably chosen from those corresponding to the formula A-Y' in which A represents a group chosen from:  , -NH-, -O-CO-NH-, S and O, and Y' represents a molecule corresponding to the formula $-(CH_2)_t-(CF_2)_rF$, in which r and t represent two integers with: $12 \geq r+t \geq 4$, such as, for example:

$-(CF_2)_4F$; $-(CF_2)_5F$; $-(CF_2)_6F$; $-(CF_2)_7F$; $-(CF_2)_8F$; $-(CF_2)_9F$; $-(CF_2)_{10}F$; $-(CF_2)_{11}F$; $-(CF_2)_{12}F$; $-(CF_2)_{13}F$; $-(CF_2)_{14}F$; $-CH_2-(CF_2)_3F$; $-CH_2-(CF_2)_4F$; $-CH_2-(CF_2)_5F$; $-CH_2-(CF_2)_6F$; $-CH_2-(CF_2)_7F$; $-CH_2-(CF_2)_8F$; $-CH_2-(CF_2)_9F$; $-CH_2-(CF_2)_{10}F$; $-CH_2-(CF_2)_{11}F$; $-CH_2-(CF_2)_{12}F$; $-CH_2-(CF_2)_{13}F$; -

$(CH_2)_2-(CF_2)_2F$; $-(CH_2)_2-(CF_2)_3F$; $-(CH_2)_2-(CF_2)_4F$; $-(CH_2)_2-(CF_2)_5F$; $-(CH_2)_2-(CF_2)_6F$; $-(CH_2)_2-(CF_2)_7F$; $-(CH_2)_2-(CF_2)_8F$; $-(CH_2)_2-(CF_2)_9F$; $-(CH_2)_2-(CF_2)_{10}F$; $-(CH_2)_2-(CF_2)_{11}F$; $-(CH_2)_2-(CF_2)_{12}F$; $-(CH_2)_3-(CF_2)_1F$; .....$-(CH_2)_{11}-(CF_2)F$. Preferably, $t \geq 2$. Preferably, $12 \geq r \geq 4$, even more preferably $10 \geq r \geq 6$.

A subject of the invention is also any biologically active molecule containing a fragment of formula (II)



(II)

in which R, AA_1, AA_2, AA_3, a_2, a_3, Y, X and x have the same definition as in formula (I) above.

In fact, the invention provides a molecule fragment of formula (II) to which it is possible to attach, by means of a suitable linkage, an active principle of any nature, as disclosed above, so as to promote the penetration of this active principle into the human or animal organism and so as to allow this active principle to reach its biological target.

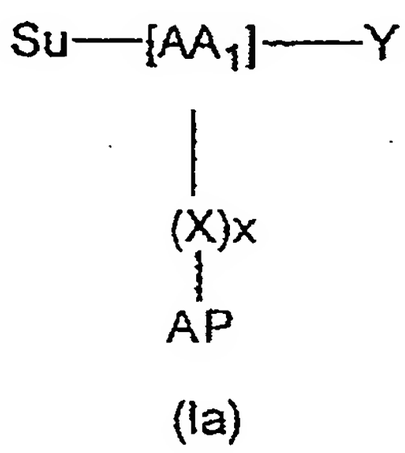
Specifically, the amphiphilic nature of the molecule promotes the passing through membranes, and the optional presence of an agent for specific recognition of the target with which the active principle is associated promotes its delivery to this target.

A subject of the invention is therefore also the use of a molecule fragment of formula (II) as defined above, for promoting the bioavailability of an active agent.

The preparation of the molecules of formula (I) is

illustrated below by means of examples corresponding to several variants of the invention. More generally, use is made of methods of protection, deprotection and coupling of peptide synthesis, which methods are well known to those skilled in the art and are disclosed in particular in the work "The peptides" Gross and Meienhofer, 3 vols, *Academic Press*, New York, 1979-1981.

Among the molecules corresponding to formula (I), one of the particular subjects of the invention consists of the molecules corresponding to formula (Ia) below:



(Ia)

in which Su represents a variant of the group R, chosen from a monosaccharide, an aminated monosaccharide derivative, a polysaccharide, a polyol or, optionally, a polyether, as were defined above;

AA_1 represents an amino acid carrying an acid, amine, alcohol or thiol function on its side chain, by means of which it is attached either to $(X)_x$-AP or to Y; AA_1 is attached to Su and either to $(X)_x$-AP, or to Y, via its N- and C-terminal ends.

X, x, AP and Y have the same definition as in formula (I) above. Y is attached to the amino or acid end of AA_1 or, optionally, to its side chain.

Preferably, one or more of the conditions below are verified:

- Su represents a monosaccharide or a polysaccharide;
- X represents a spacer arm that is peptide in

nature, containing at least one tyrosine residue; preferably, X represents tyrosine;

- AA_1 represents an amino acid chosen from arginine and lysine;

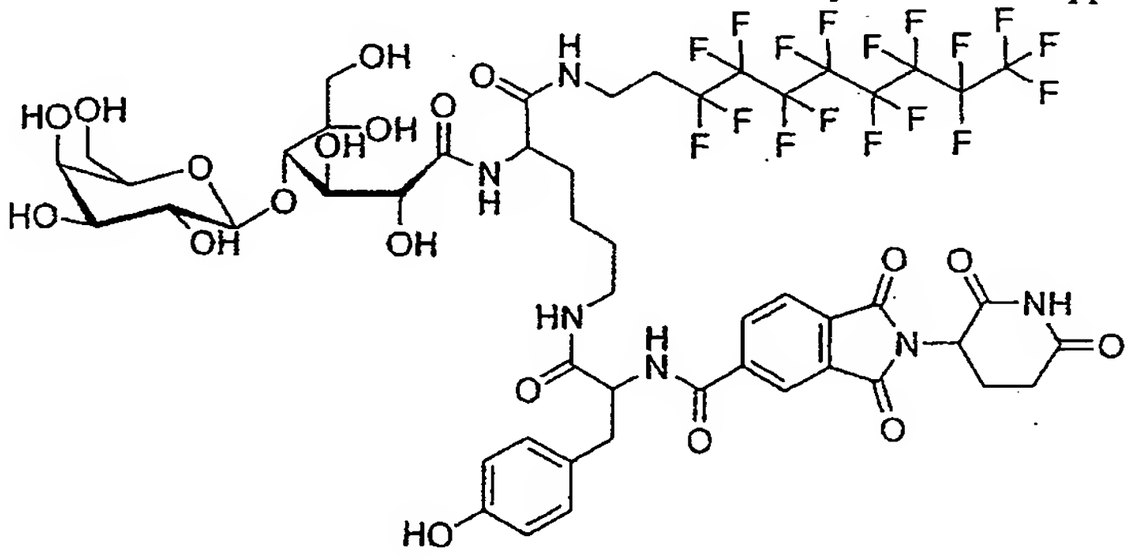
- Y represents a fluorinated C_6-C_{12} hydrocarbon-based chain containing from 5 to 23 fluorine atoms, attached to the amino acid AA_1 via an -NH- function.

Two examples of compounds of formula (Ia) are illustrated below and in the examples:

a) Example 1: Targeting of angiogenic sites

Angiogenesis is a natural biological process for creating new blood microvessels from pre-existing venules. It is a complex phenomenon that occurs normally in adults only under certain specific conditions such as wound healing, inflammation or the development of the corpus luteum during the menstrual cycle. Under normal conditions, the process of angiogenesis stops after an appropriate amount of time, indicating correct regulation of the stimulatory and inhibitory factors. Under certain pathological conditions, such as solid tumor growth, rheumatoid arthritis, psoriasis or diabetic retinopathy, angiogenesis develops in a clearly less controlled manner ("Antiangiogenic agents and their promising potential in combined therapy", P.A. Burke, S.J. DeNardo, *Crit. Rew. In Oncology/Hematology*, (2001), **39**, 155-171). More than 30 years ago, J. Folkman put forward the hypothesis that solid tumor growth was closely linked to the development of angiogenesis, and since then, a very large number of teams have shown an interest in this phenomenon and have tried to develop substrates capable of blocking the angiogenic process ("Tumor angiogenic therapeutic applications" J. Folkman *Engl. J. Med.* (1971), **285**, 1182-1186 and "Tumor angiogenis past, present and the near future". R.S. Kerbel *Carcinogenesis* (2000), **21**, 505-521). Among the various structures tested, thalidomide, initially

prescribed to pregnant women as a sedative and responsible for problems of teratogenesis, proved to be extremely advantageous for inhibiting vascular development. The idea that prevailed in the work carried out was to graft thalidomide onto the vector provided beforehand with a radioactive unit such as iodine 125-labeled tyrosine. The aim being sought in this example is to readily visualize the angiogenic sites *in vivo*, and therefore the solid tumors, and to block their development.



Molecule model A

With this aim, a central lysine unit was provided with a fluorocarbon chain on the primary acid function, with a unit of lactose type capable of providing the molecule with the water-solubility necessary for its intravenous or intraperitoneal administration, and with tyrosine that is subsequently labeled with iodine 125 and onto which thalidomide, provided beforehand with a reactive acid function in the 3-position, is grafted.

According to a preferred variant of the invention, in the molecules corresponding to formula (Ia), the active principle is chosen from molecules capable of blocking the angiogenic process, in particular thalidomide.

b) Example 2: Spin-trap vectorization

Mitochondrial cytopathies include a large variety of diseases, the common denominator of which is a deficiency in the mitochondrial respiratory chain. Due

to the ubiquitous presence of mitochondria in the organism, this dysfunction can affect any organ. The effect can be isolated or, on the contrary, plurivisceral, in that case generally showing dominance in the neuromuscular system. No treatment currently exists for these diseases, which can be classified within the context of "orphan diseases".

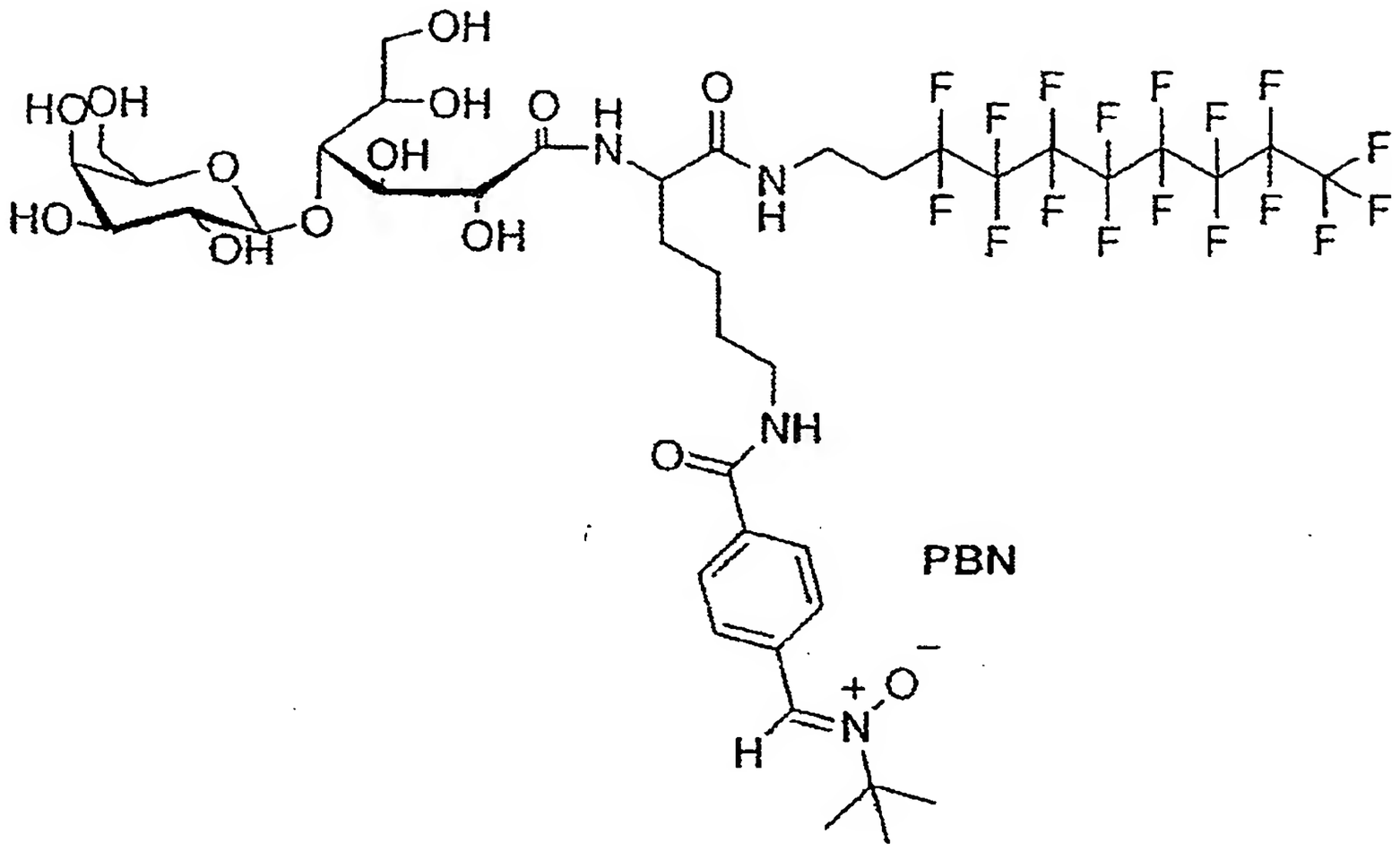
It is, however, now clear that, since mitochondria are, in the cell, the preferred site for the production of free radicals, deficiencies in the respiratory chain are very commonly associated with an overproduction of free radicals, the consequence of which is accelerated cell death in the affected tissues. Recent studies carried out at the Necker hospital in the team of Dr. P. Rustin ("Increased apoptosis *in vivo* in cells lacking mitochondrial DNA gene expression", Wang J, Silva JP, Gustafsson C, Rustin P, Larsson NG. *Proc Natl Acad Sci USA* (2001) (in press)) have made it possible to show, on a series of human cells in culture, the importance of this production of free radicals of oxygen. These cell cultures represent all the types of deficiencies affecting the various complexes of the respiratory chain that are known in humans. They were characterized both from the point of view of the deficiency affecting the respiratory chain and from the point of view of the production of free radicals and of its consequences on cell survival. This collection of cells represents an irreplaceable tool for studying the effectiveness of any molecule whose target is the free-radical reactions associated with respiratory chain deficiencies.

The recent identification, in our team, of a "spin-trap" molecule capable of blocking cell death in cell models of apoptosis induced by free radicals produced by the respiratory chain has given us a basis for developing similar molecules exhibiting further enhanced effectiveness ("Superoxide-induced massive

apoptosis in cultured skin fibroblasts harboring the Neurogenic Ataxia Retinitis Pigmentosa (NARP) mutation in the ATPase-6 gene of the mitochondrial DNA". Geromel V, Kadhom N, Ceballos-Picot I, Ouari O, Polidori A, Munnich A, Rötig A, Rustin P. *Hum Mol Genet* (2001) (in press)). The aim pursued here was to refine and to simplify the structure of these substrates while at the same time conserving their biological activity in order to develop a synthetic process that can be readily adapted to the industrial phase. The tests were carried out on several cell models: cell cultures exhibiting a deficiency in the mitochondrial respiratory chain (fibroblasts in culture), neuron/muscle cell cocultures subjected to the action of free radicals and, finally, on cells extracted from skin having been subjected to a 3rd degree burn.

The purpose of the research undertaken was to have free-radical traps that can be used clinically for treating apoptotic phenomena, and more generally cell death phenomena attributable to the overproduction of free radicals. The extremely encouraging results obtained on these cell types fully justify the development of these amphiphilic vector models.

Molecule **E**, constructed on the previous molecule, was provided, in this particular case, with a well-known and effective spin-trap that contains a derivative of PBN.



Molecule **E**

The first tests were carried out *in vitro* at the Necker hospital on fibroblasts derived from a biopsy of skin from a child exhibiting the NARP mutation. In a similar manner to the product TA1PBN ("Superoxide-induced massive apoptosis in cultured skin fibroblasts harboring the Neurogenic Ataxia Retinitis Pigmentosa (NARP) mutation in the ATPase-6 gene of the mitochondrial DNA", Geromel V, Kadhom N, Ceballos-Picot I, Ouari O, Polidori A, Munnich A, Rötig A, Rustin P. *Hum Mol Genet* (2001) (in press) and "Synthesis of a glycolipidic amphiphile nitrone as a new spin trap for biological applications" O. Ouari, A. Polidori, F. Chalier, P. Tordo, B. Pucci. *J. Org. Chem.*, (1994), **64**, 3554-3556) previously tested, molecule **E** exhibits a capacity for cell protection and inhibits the apoptotic process. No toxicity was measured, with respect to this type of product, on any of the cells placed in culture.

These results validate once again the advantage of such a vectorization concept and clearly show its potentialities in fields of application that are entirely different.

According to another preferred variant of the invention, in the molecules corresponding to formula (Ia), the active principle is chosen from free-radical scavengers, in particular N-benzylidene-tert-butylamine oxide derivatives.

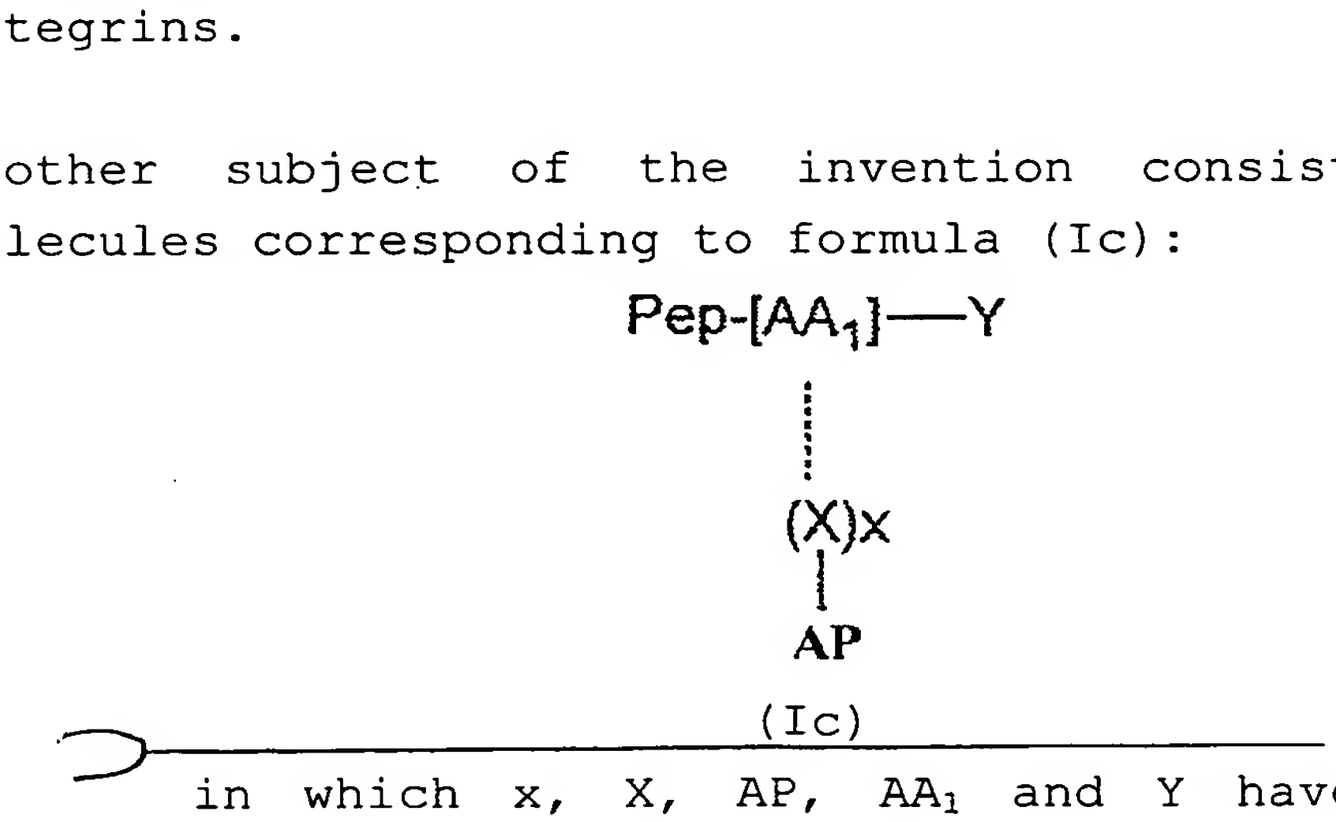
Among the molecules corresponding to formula (II), another particular subject of the invention consists of the molecules corresponding to formula (Ib):

$$Pep\text{-}[AA_1]\text{-}Y \qquad (Ib)$$

in which Y and AA_1 have the same definition as in formula (I) above, in particular as in formula (Ia), and Pep, which is a variant of R, represents a peptide chain containing from 2 to 10, preferably from 4 to 6, amino acids. Advantageously, Pep or AA_1 contains at least one tyrosine unit.

Advantageously, Pep is chosen for its affinity for a given biological target; in particular, this peptide chain can contain an RGD (arginine-glycine-aspartic acid) sequence that is known to be recognized by $\alpha V\beta 3$ integrins.

Another subject of the invention consists of the molecules corresponding to formula (Ic):



(Ic)

in which x, X, AP, AA_1 and Y have the same definition as in formula (I) above; in particular, the molecules in which x, X, AP, AA_1 and Y have the same definition as in formula (Ia) above; Pep has the same

definition as in formula (Ib) above.

Preferably, one or more of the conditions below are verified:

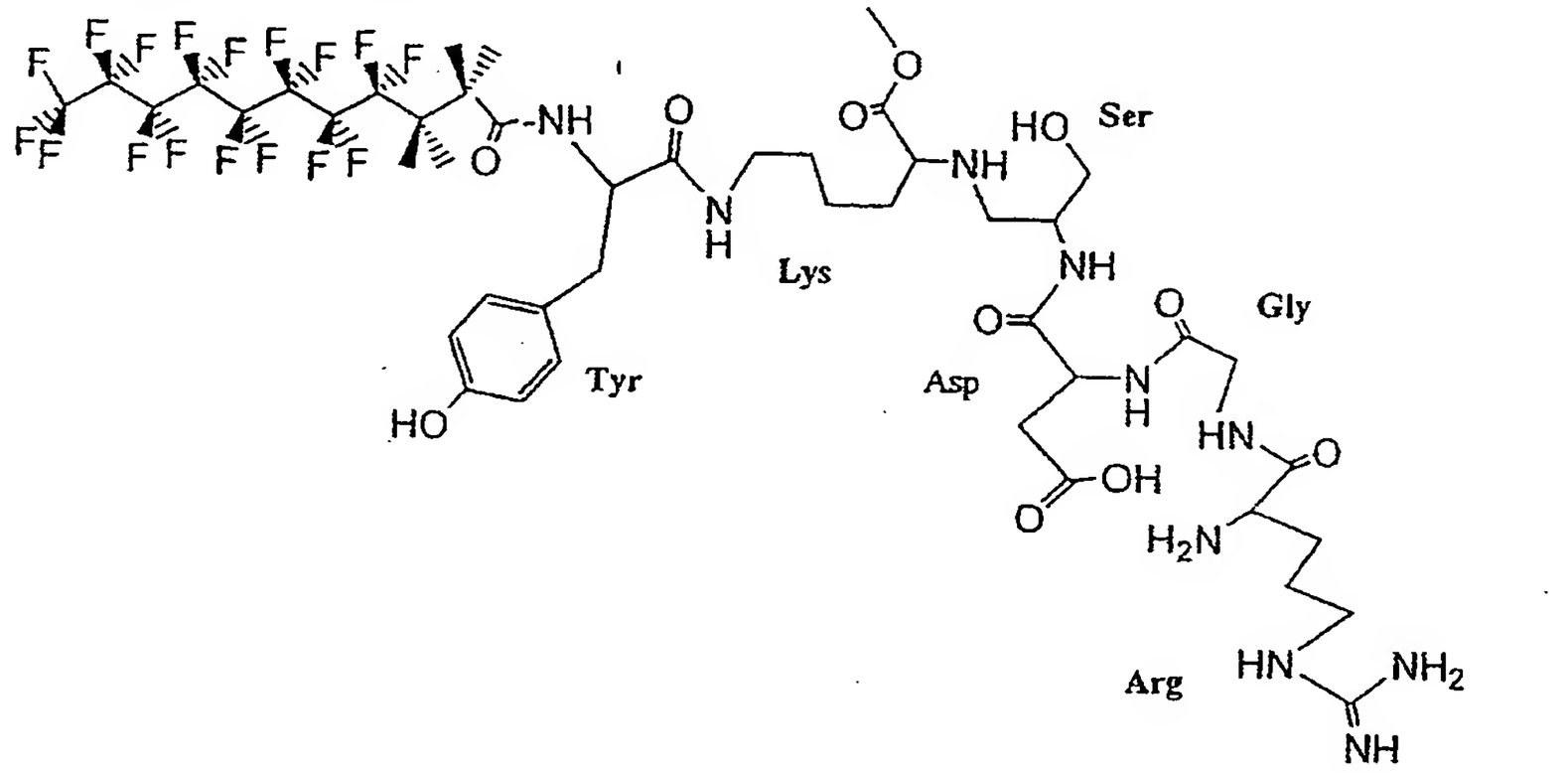
- Pep is a peptide recognized by αVβ3 integrins and AP is an antimitotic agent;
- X, Pep or AA_1 contains at least one tyrosine residue;
- X represents a chain of 1 to 3 amino acids.

An example of compounds of formulae (Ib) and (Ic) is illustrated below and in the experimental section:

c) Example 3: Anticancer therapy.

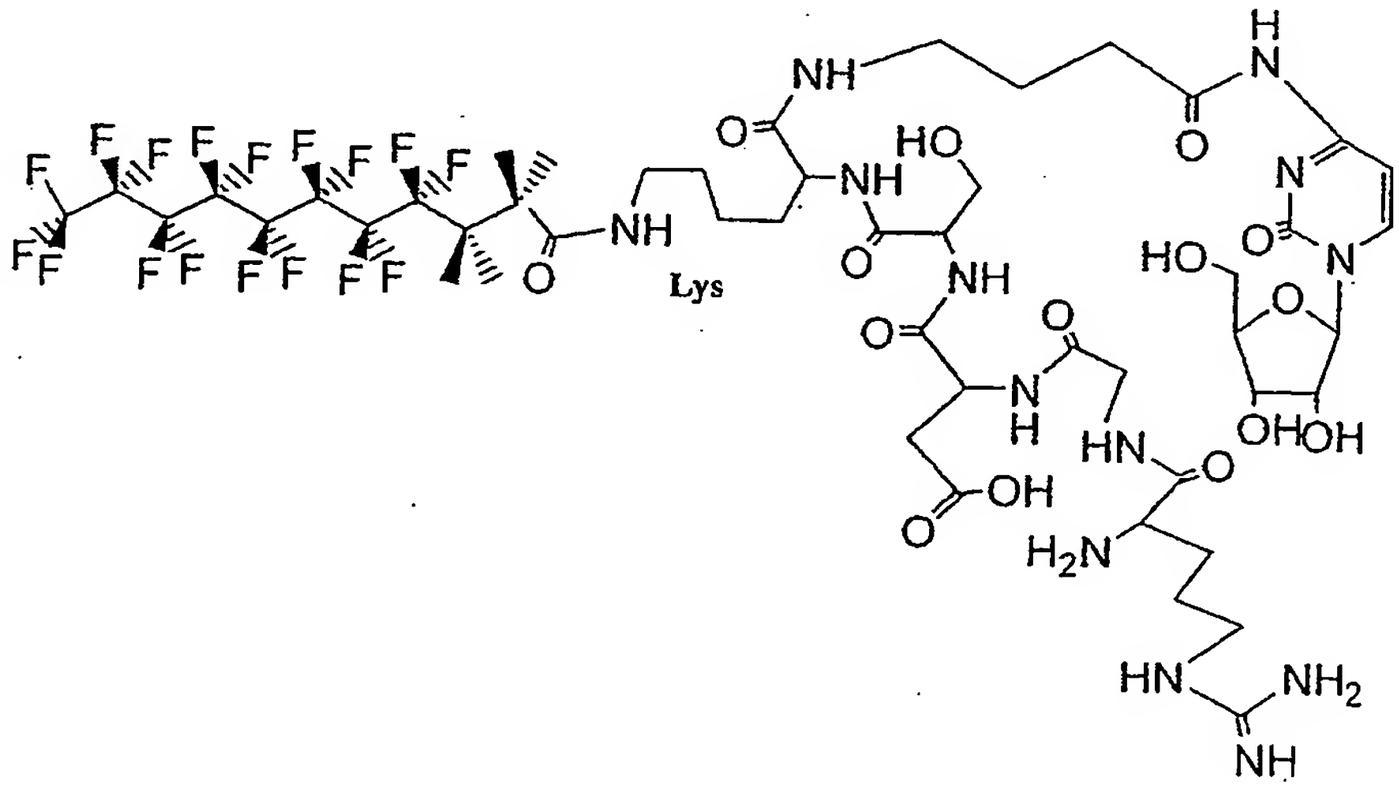
At the current time, no technique has made it possible to demonstrate a criterion for effective differentiation, and therefore for appropriate targeting, of tumor cells and normal cells. As has been seen, however, the growth of a cancerous tumor is closely linked to its rate of vascularization and therefore to the phenomenon of angiogenesis that accompanies it. These observations had the scientific community to develop substrates, as has been seen, capable of inhibiting angiogenesis and therefore of blocking, by this means, the development of tumors. It is in fact now commonly accepted that membrane proteins carried by the angiogenic cells, and called integrins, participate actively in the process of proliferation of these cells. More particularly, αVβ3 integrins recognize a specific peptide sequence, the RGD (arginine-glycine-aspartic acid) sequence. The grafting of this unit onto the proposed vector should therefore provide it with an ability to specifically target angiogenic sites and therefore, ultimately, tumor sites. The addition, to this same vector, of an antimitotic agent should allow the selective destruction of the cancerous cells. In this perspective, molecule **B** was initially prepared to verify the innocuousness of the vector and to determine

its specificity. In order to give the targeting agent a greater degree of freedom, said targeting agent was grafted onto the central lysine by means of a hydrophobic amino acid, serine. The molecule is water-soluble and is amphiphilic in nature.

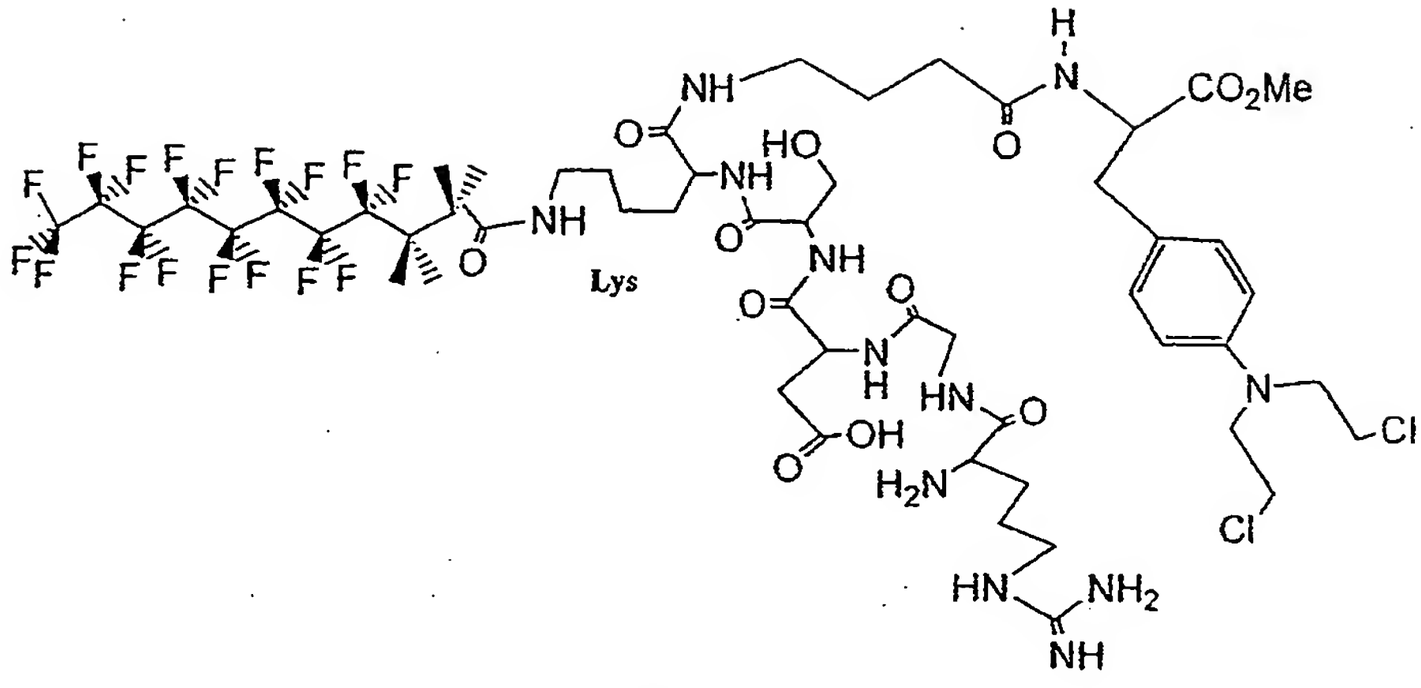


Structure of molecule **B**

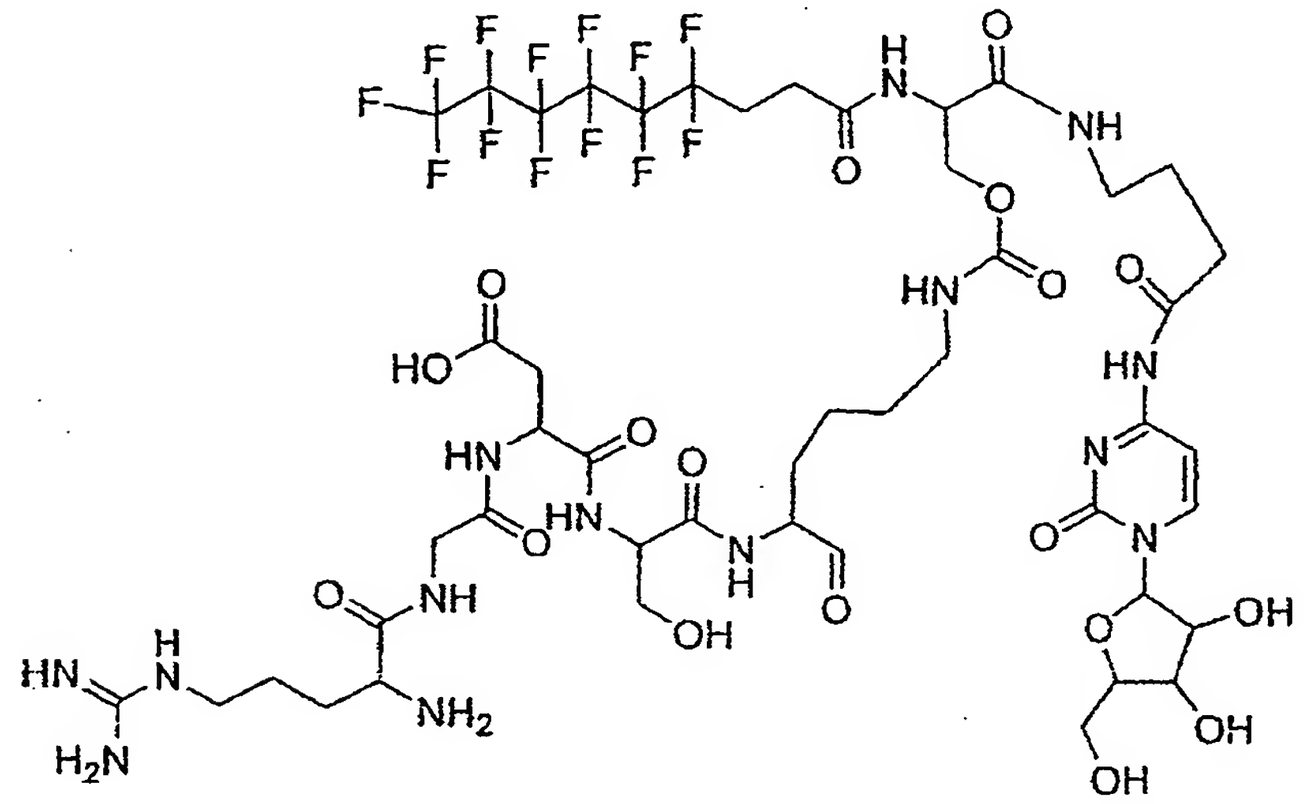
Given these positive results, further vectors carrying the RGD peptide sequence and an antimitotic agent such as melphalan (molecule **D**) or Ara-C (molecules **C** and **F**) were then synthesized and are in the process of being analyzed for their anticancer activity.



Molecule **C** (Ara-C)



Molecule **D** (Melphalan)



Molecule **F**

Structure of vectors C, F and D

The antimitotic agents selected are merely models here, and simply illustrate the convenience of introducing and transporting a given antimitotic agent. This type of vector can and will also be used as an agent for the vectorization of substrates such as adriamycin (in this specific case, the molecule (Ic) contains a peptide fragment, either at X or at Pep, of Gly-Phe-Leu-Gly type), of 5-Fu (5-fluorouracil), of Melphalan, or of tyrosine kinase inhibitors such as imatinib mesylate (STI571, Glivec®), for example, or more generally of any anticancer agent that can be grafted onto these carriers. The presence of the hydrophobic fluorocarbon chain promotes the transmembrane passage. The release

of the active principle is provided by hydrolysis of the peptide linkages by means of the appropriate cytoplasmic enzymes.

The first *in vitro* results already obtained fully validate this concept.

A subject of the invention is also the use of the compounds corresponding to formula (I) as defined above, for preparing a medicinal product.

In fact, it has been demonstrated that the compounds corresponding to formula (I) according to the present invention have a bioavailability and an ability to reach their biological targets that are greater than or equal to those of the compounds of the prior art.

This property makes it possible to envision the use of the molecules of the invention in varied fields:

- in the therapeutic field, the products of the invention can be used for the prevention and/or treatment of all kinds of pathologies, in particular the various forms of cancer, and pathologies associated with oxidative stress and with the formation of oxygenated free-radical species.

Consequently, a subject of the invention is the pharmaceutical compositions comprising a compound according to the invention in a pharmaceutically acceptable carrier.

A subject of the invention is also the use of a compound of formula A, C, D or F, for preparing a pharmaceutical composition intended to prevent and/or treat cancer.

A subject of the invention is also the use of a compound of formula B, for preparing a pharmaceutical

composition intended to detect the presence of cancerous cells.

A subject of the invention is also the use of a compound of formula E, for preparing a pharmaceutical composition intended to prevent and/or treat pathologies associated with oxidative stress and with the formation of oxygenated free-radical species, in particular immune and inflammatory diseases, ischemia-reperfusion syndrome, arteriosclerosis, Alzheimer's disease, Parkinson's disease, lesions due to UV and ionizing radiation, certain cancers such as melanomas, and cell aging.

The products of the invention can be administered by any route known to those skilled in the art, in particular by intravenous or intramuscular injection, or by oral or cutaneous administration. They can be used alone or in combination with other active agents. The dose thereof and the amount administered daily are adapted according to the activity measured for the molecule concerned and according to the weight of the patient;

- in the cosmetics field, the compound of formula E can be used to prevent and/or treat the effects of aging.

A subject of the invention is therefore also a cosmetic composition comprising a compound of formula E in a cosmetically acceptable carrier.

Said composition may be intended for application to the skin or to the integuments (nails, hair).

It may be in the form of an aqueous or oily solution, of a water-in-oil or oil-in-water emulsion, of a triple emulsion or of an ointment.

The compounds of the invention can be introduced into any cosmetic composition for which a free-radical scavenger activity is desired: a skincare cream, an antisun product, a makeup-removing product, a mask for the skin or the hair, a shampoo, a makeup product such as a lipstick, a blusher, a foundation, a nail varnish, etc.

Due to their solubility in varied media, the compounds of the invention are easy to use and can be employed under very diverse conditions.

EXPERIMENT SECTION

1/ Example 1:

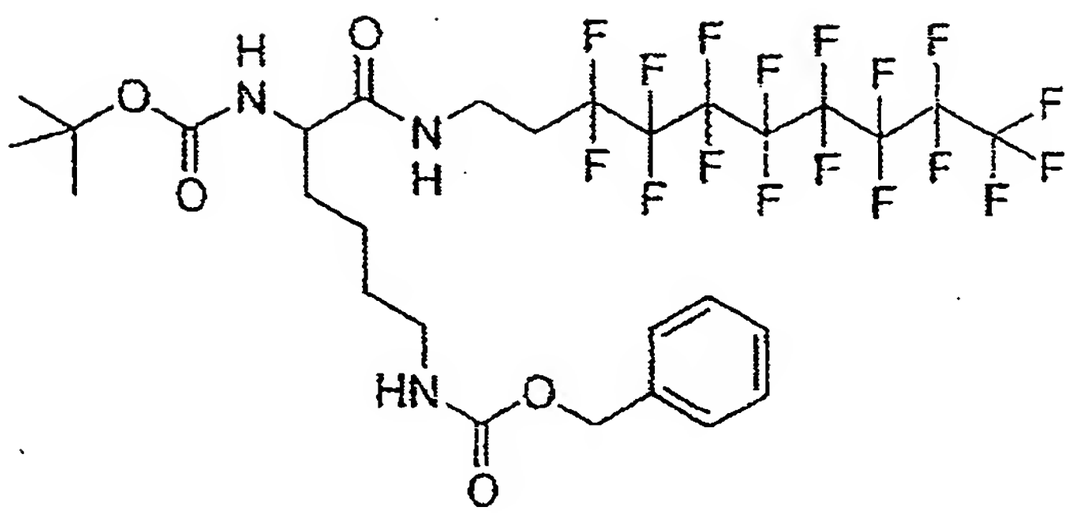
A- Preparation of molecule A

2 g (4 mmol) of 1H,1H,2H,2H-perfluorodecane azide compound dissolved in 30 ml of anhydrous methanol are subjected to hydrogenation in the presence of palladium-on-charcoal. After reaction for 4 hours, the medium is filtered through celite and the solvent is evaporated off under reduced pressure. The corresponding amine **2** is isolated without purification (quantitative yield).

The compound **2** is reacted again, in 30 ml of dichloromethane, in the presence of 2.2 g (4 mmol) of Boc-Lys-(Z)-$OPhF_5$ **3**. The pH of the solution is brought to 8 by adding a few drops of DIEA.

After stirring for 16 hours at ambient temperature, the reaction medium is concentrated under reduced pressure.

The medium is purified by silica gel column chromatography (eluant: 3/7 ethyl acetate/cyclohexane). By means of crystallization from an ethyl acetate/hexane mixture, the fluorinated compound **4** (2.68 g; 3.24 mmol; 80%) is obtained in the form of a white powder.



4

rf: 0.36 in 5/5 cyclohexane/ethyl acetate.

$[\alpha]_D$ = -8.2 (c: 1; $CHCl_3$).

Melting point: 86.5-88.3°C.

^1H NMR (250 MHz, $CDCl_3$): δ 7.36 (5H, s, CH arom), 6.85 (1H, m, NH amide), 5.27 (1H, d, J = 6.65 Hz, NH urethane), 5.11 (2H, s, CH_2O), 4.99 (1H, t, J = 5.8 Hz, NH urethane), 4.06 (1H, m, CHCO), 3.58 (2H, dd, J = 6.5 Hz, CH_2NH), 3.20 (2H, dd, J = 6.2 Hz, CH_2NH), 2.35 (2H, m, $\underline{C}H_2CF_2$), 2.0 to 1.0 (15H, m, CH_3 from Boc and CH_2 Lys).

^{13}C NMR (62.86 MHz, $CDCl_3$): δ 172.5 (CONH), 156.7; 155.9 (OCONH), 136.6 (C^{IV} arom.), 128.5; 128.1 (CH arom.), 80.4 (C^{IV}), 66.7 (CH_2OCONH), 54.4 (CHCO), 40.2 (CH_2NH), 31.9 (CH_2NH), 31.3 (CH_2), 30.7 (CH_2Rf), 29.5 (CH_2), 28.2 (CH_3 from tert-butyl), 22.4 (CH_2).

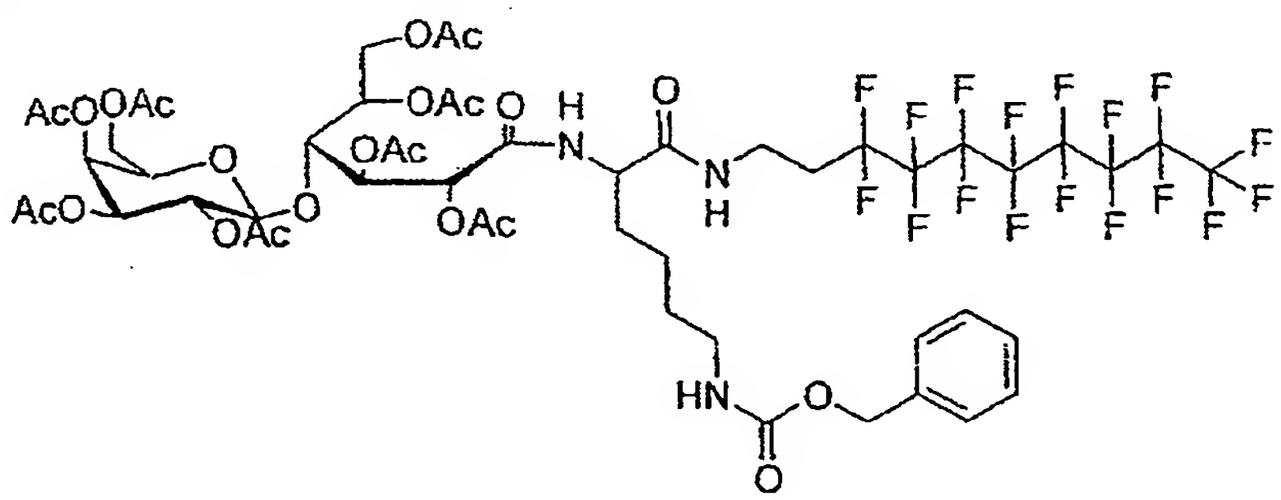
^{19}F NMR (235 MHz, $CDCl_3$): δ -80.7 (3F, s, CF_3), -113.9 (2F, s, CF_2CH_2), -121.9 (6F, s, $(CF_2)_3$), -122.7 (2F, s, CF_2), -123.5 (2F, s, CF_2), -126.6 (2F, s, $\underline{C}F_2CF_3$).

0.5 g (0.6 mmol) of compound **4** dissolved in 30 ml of dioxane is subjected to hydrogenation in the presence of palladium-on-charcoal.

After reaction for 15 hours, the medium is filtered through celite and the solvent is evaporated off under reduced pressure. The amine **5** obtained is reacted in dichloromethane in the presence of 0.21 g (0.44 mmol) of freshly prepared lactobionolactone and DIEA is added

to the medium in order to bring the pH of the solution to 8.

After complete disappearance of the amine **5** (TLC), the reaction medium is concentrated under reduced pressure. 40 ml of a 1:1 acetic anhydride/pyridine mixture are added, under cold conditions, to the reaction crude. The stirring is maintained at ambient temperature for 18 hours and the reaction mixture is then poured onto 150 ml of 1N HCl. The aqueous phase is extracted 3 times with 50 ml of dichloromethane. The organic phase is respectively washed twice with 60 ml of 1N HCl and then with 60 ml of brine and, finally, dried over Na_2SO_4. The solvents are eliminated under reduced pressure and the crude is purified by flash chromatography on silica gel (eluant: 6/4 then 7/3 ethyl acetate/cyclohexane) so as to produce the compound **6** (0.55 g; 0.39 mmol; 65%) in the form of a white powder.



6

rf: 0.22 in 6/4 ethyl acetate/cyclohexane

$[\alpha]_D$ = +2.9 (c, 1; $CHCl_3$).

Melting point: 65°C (beginning of decomposition).

^1H NMR (250 MHz, DMSO-d6): δ 8.07 (2H, m, NH), 7.34 (5H, s, CH arom.), 7.01 (1H, m, NH), 5.47 (1H, m, H from the sugar), 5.30 to 5.10 (2H, m, H from the sugar), 5.02 to 4.79 (5H, m, CH_2-O and H from the sugar), 4.50 to 3.90 (8H, m, H from the sugar and CHα from lysine), 3.38 (2H, m, CH_2-NH), 2.97 (2H, m, CH_2-NH), 2.30 (2H, m, CH_2-CF_2), 2.14, 2.09, 2.04, 2.01,

1.96, 1.92 (24H, 6s, CH_3 from acetyls), 1.65 to 1.10 (6H, m, CH_2 from lysine)

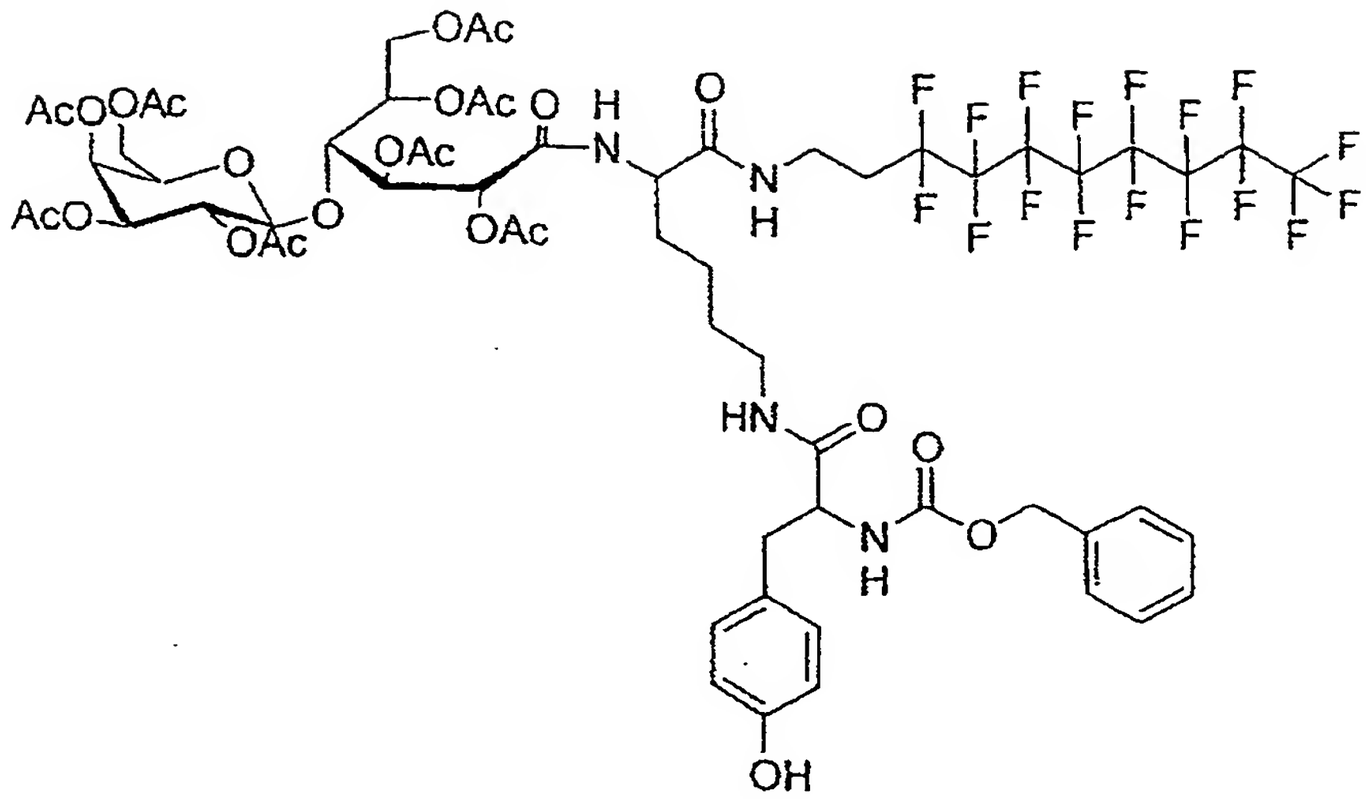
^{13}C NMR (62.86 MHz, $CDCl_3$): δ 171.3 (CO-NH), 170.5, 170.5, 170.1, 170.0, 170.0, 169.7, 169.2 (7s, CO-O), 167.9 (CO-NH), 156.7 (O-CO-NH), 136.7 (C^{IV} arom.), 128.4, 128.0, 127.9 (CH arom.), 101.6 (CH-1'), 77.9 (CH-4), 72.7 (CH-2), 71.1, 70.9 (CH-5' and CH-3'), 70.0 (CH-5), 69.4 (CH-3), 69.0 (CH-2'), 66.9 (CH-4'), 66.5 (CH_2-O), 61.6, 61.0 (CH_2-6 and CH_2-6'), 52.6 (CH-CO), 40.4 (CH_2-NH), 31.9 (CH_2-NH), 31.2 (CH_2-), 30.5 (CH_2-Rf), 29.1 (CH_2-), 22.2 (CH_2-), 20.6, 20.5, 20.4, 20.3 (CH_3 from acetyls)

^{19}F NMR (235 MHz, DMSO-d6): δ -80.2 (3F, s, CF_3), -113.0 (2F, s, $\underline{CF_2}$-CH_2), -121.4 (6F, s, $3CF_2$), -122.2 (2F, s, CF_2), -123.0 (2F, s, CF_2), -125.4 (2F, s, CF_2-CH_2).

The benzyloxycarbonyl group of compound **6** is deprotected according to the experimental protocol already described when passing from compound **4** to compound **5**. Using 0.5 g (0.36 mmol) of compound **6**, the amine **7** is obtained with a quantitative yield.

The amine **7** obtained is reacted in 30 ml of dichloromethane in the presence of 0.21 g (0.44 mmol) of Z-Tyr-$OPhF_5$ (compound **8**) and DIEA is added in order to bring the pH of the solution to 8.

After complete disappearance of the amine **7** (TLC), the reaction medium is concentrated under reduced pressure and purified by silica gel column chromatography (eluant: 7 ethyl acetate/3 cyclohexane). The compound **9** (0.32 g; 0.21 mmol; 58%) is obtained in the form of a white powder.



rf: 0.35 in 7 ethyl acetate/3 cyclohexane.

$[\alpha]_D$ = +1.5 (c: 1; $CHCl_3$).

Melting point: 37.6°C (beginning of decomposition).

^1H NMR (250 MHz, DMSO-d6): δ 8.01 (2H, m, NH), 7.71 (1H, m, NH), 7.21 (5H, s, CH arom.), 7.05 (3H, m, NH, 2H arom tyr), 6.89 (2H, d, J = 8.29 Hz 2H arom tyr), 5.22 (1H, d, J = 5.35 Hz H from the sugar), 5.02 (2H, s, CH_2Ph), 4.99 (2H, s, CH_2Ph), 4.92 (2H, m, H from the sugar), 4.70 to 4.55 (5H, m, H from the sugar), 4.01 to 3.75 (9H, m, CHα tyr, CHα lys, CH_2NH, 5H from the sugar), 2.76 (2H, m, CH_2-NH), 2.16 (2H, m, CH_2-CF_2), 1.90 to 1.68 (24H, 6s, CH_3 from acetyls), 1.29 to 0.91 (6H, m, CH_2 from lysine)

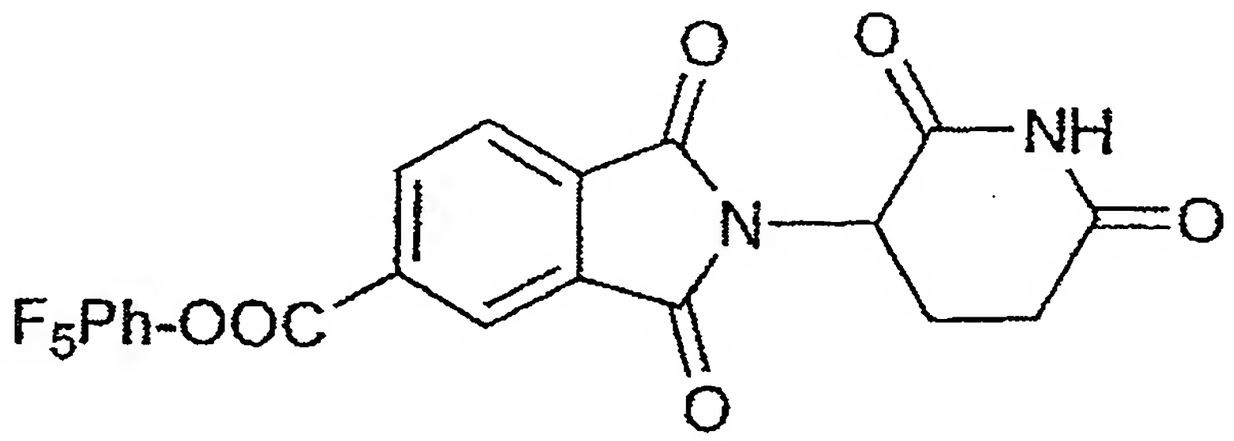
^{13}C NMR (62.86 MHz, $CDCl_3$): δ 171.85; 171.48 (CO-NH), 170.67, 170.32, 170.18, 170.0, 169.63; 169.42 (6s, CO-O), 156.29 (CO-NH), 137.47; 136.61; 135.54 (C^{IV} arom.), 130.71; 129.03; 128.80; 128.71; 128.12; 127.96; 121.17 (CH arom.), 101.07 (CH-1'), 78.69 (CH-4), 72.22 (CH-2), 70.86; 70.21 (CH-5' and CH-3'), 70.06 (CH-5), 69.61 (CH-3), 69.3 (CH-2'), 67.60 (CH-4'), 65.69 (CH_2-O), 61.72, 61.48 (CH_2-6 and CH_2-6'), 56.62 (CHαtyr); 52.68 (CHαlys); 37.48 (CH_2-NH), 31.86 (CH_2-NH), 31.43 (CH_2-), 30.08 (CH_2-Rf), 29.09 (CH_2-), 22.77 (CH_2-), 21.08, 21.03, 20.94, 20.86, 20.76 (CH_3 from acetyls)

^{19}F NMR (235 MHz, DMSO-d6): δ -80.19 (3F, s, CF_3), -113.38 (2F, s, $\underline{CF_2}$-CH_2), -121.67 (6F, s, $3CF_2$),

-122.45 (2F, s, CF_2), -123.24 (2F, s, CF_2), -125.70 (2F, s, CF_2-CH_2).

Once again, according to the experimental protocol already described, 0.3 g (0.19 mmol) of compound **9** dissolved in 30 ml of ethanol is subjected to hydrogenation in the presence of palladium-on-charcoal.

The amine **10** obtained is reacted in dichloromethane in the presence of 0.107 g (0.23 mmol) of active ester of thalidomide **11** and DIEA is added in order to bring the pH of the solution to 8.



11

Rf= 0.65 in 7 EtOAc/3 cyclohex.

$[\alpha_D]$ = +3.9 (c: 1; DMF).

^1H NMR (250 MHz, $CDCl_3$): 8.63 (1H, s, Ph); 8.61 (1H, d, Ph); 8.09 (1H, d, Ph); 5.04 (1H, m, NC<u>H</u>); 2.86 (3H, m, C<u>H</u>$_2$CO, C<u>H</u>CH$_2$CO); 2.19 (2H, m, CHC<u>H</u>$_2$).

^{19}F NMR (235 MHz, $CDCl_3$): -152.6 (2F, d, CF); -156.67 (1F, t, CF); -161.87 (2F, t, CF).

^{13}C NMR (62.86, DMSO): 177.97; 174.93; 171.64; 171.61; 170.96 (5 CO); 142.01; 140.94; 139.68; 136.89; 129.09; 128.64 (aromatic Cs); 54.44 (N<u>C</u>H); 36.13 (C<u>H</u>$_2$CO); 27.12 (NCH<u>C</u>H$_2$)

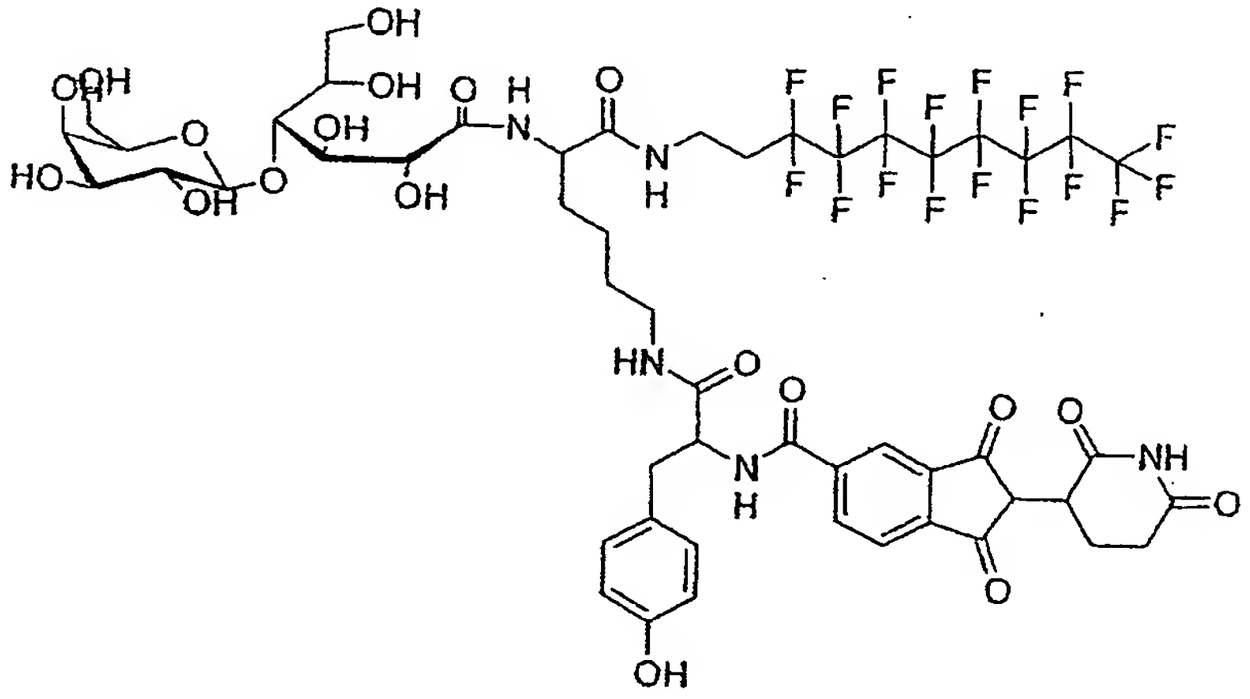
After complete disappearance of the amine **10** (TLC), the reaction medium is concentrated under reduced pressure and purified by silica gel column chromatography (eluant: 8/2 to 9/1 ethyl acetate/cyclohexane).

The compound **12** is obtained with a very low yield (11 mg; 6.4 µmol; 4.5%).

rf: 0.63 in ethyl acetate.

The deacetylation of the saccharide portion of the molecule is carried out at ambient temperature in methanol containing a catalytic amount of sodium methoxide.

After treatment on H^+ resin (amberlite IRC 50), filtration and evaporation of the solvent, the deacetylated product **A** is isolated with a quantitative yield.



A

B- Biological assays

Such a substrate exhibits no totally unacceptable toxicity on cell cultures of fibroblasts and of B16 melanomas. *In vivo*, the molecule is concentrated in the stroma of the tumor, which makes it possible to visualize it very clearly (cf. Table 1).

Tissue	15 min	1 h
Tumor (stroma)	4.5 ± 0.7	5.4 ± 0.2
Tumor (center)	2.6 ± 0.5	2.7 ± 0.5
Blood	5.9 ± 1.2	3.4 ± 0.7
Liver	4.5 ± 1.1	2.2 ± 0.8
Kidney	4.6 ± 0.5	4.0 ± 0.9
Thyroid	5.6 ± 0.8	9.8 ± 1.7

Table 1: Radioactivity measured in various organs of mice carrying B16 melanomas after IP injection of molecule **A** (10 µCi/animal).

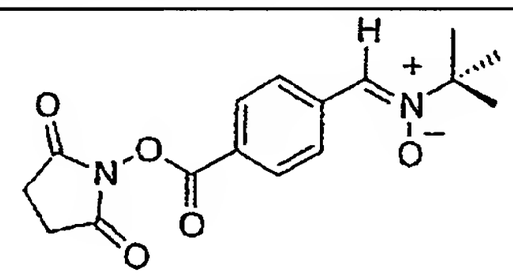
The ongoing research will make it possible to specify its effectiveness, compared with thalidomide alone, in blocking tumor development. The first results obtained in vascular growth assays on chick embryos (chick aortic ring assays) showed the effectiveness of this structure in inhibiting microvessel growth. Molecule **A** is found to be effective at 20 µM and completely blocks the development at 200 µM, whereas, at such concentrations, thalidomide is found to be ineffective.

This first result demonstrates the general innocuity of the proposed structure and its possible advantage for diagnosis, thalidomide here being merely an example of an active principle.

2/ Example 2: preparation of molecule E

This synthesis example is illustrated in figure 1.

Synthesis of the active ester HOOCPBN



Y

N-(tert-Butyl)hydroxylamine acetate is dissolved in a saturated aqueous sodium carbonate solution. The hydroxylamine is extracted with ether. The organic phase is dried over Na_2SO_4 and the solvent is then eliminated under reduced pressure so as to produce free N-*tert*-butylhydroxylamine in the form of pulverulent white crystals.

1.00 g of 4-carboxybenzaldehyde (6.67 mmol - 1 equiv.) and the tip of a spatula of 4 Å molecular sieve are suspended, under an argon atmosphere, in 5 ml of degassed anhydrous ethanol. 0.570 g of hydroxylamine (6.37 mmol - 0.95 equiv.) in solution in 5 ml of ethanol are added to the benzaldehyde solution, and the medium is brought to 60°C, in the dark. After 18 hours,

0.200 g of hydroxylamine (2.25 mmol - 0.33 equiv.) are added to the medium and stirring is continued for a further 18 hours. The reaction mixture is filtered through a layer of celite, the solvent is eliminated under reduced pressure and the crude is purified by flash chromatography on silica gel (eluant: 6:4 ethyl acetate/cyclohexane). After recrystallization from a methanol/ether mixture, the nitrone **X** (0.590 g - 2.67 mmol - 40%) is obtained in the form of a white powder.

Molar mass ($C_{12}H_{15}NO_3$): 221.3 $g \cdot mol^{-1}$

Melting point: 214.5 - 215.7°C.

^1H NMR (250 MHz, DMSO-d6): δ 8.42 (2H, m, J = 8.5 Hz, H arom.), 7.95 (3H, m, H arom. and CH=N(O)), 1.51 (9H, s, CH_3 from *tert*-butyl).

^{13}C NMR (62.86 MHz, DMSO-d6): δ 166.9 (CO), 135.3 (C^{IV} arom), 131.0 (CH=N(O)), 129.2 (CH arom.), 128.3 (C^{IV} arom), 127.9 (CH arom.), 71.1 (C^{IV}), 27.8 (CH_3 from *tert*-butyl).

UV (MeOH, nm): λ_{max} = 287.

0.260 g of nitrone **X** (1.18 mmol - 1 equiv.) is dissolved, under an argon atmosphere, in 15 ml of dioxane. 0.290 g of DCC (1.41 mmol - 1.2 equiv.) and 0.16 g of HOSu (1.41 mmol - 1.2 equiv.) are added to the reaction medium. After stirring for 48 hours, the reaction medium is filtered through sintered glass (porosity 4) and the solvent is then eliminated under reduced pressure. After purification by flash chromatography on silica gel (eluant: 6:4 ethyl acetate/cyclohexane) and then recrystallization from an ethyl acetate/hexane mixture, the compound **Y** (0.25 g - 0.79 mol - 67%) is obtained in the form of a white powder.

Molar mass ($C_{16}H_{18}N_2O_5$): 318.3 $g \cdot mol^{-1}$

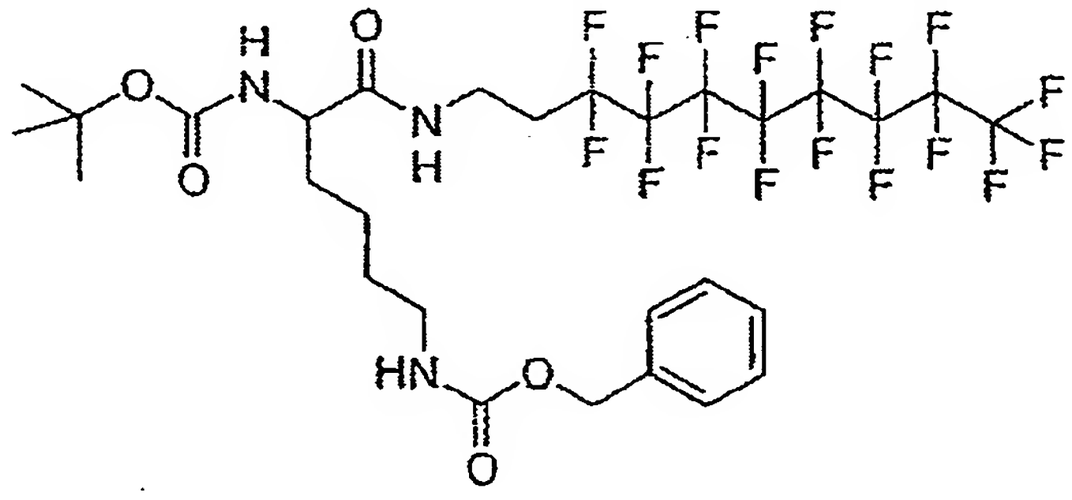
Melting point: 177.4 - 178.3°C.

^1H NMR (250 MHz, $CDCl_3$): δ 8.37 (2H, m, J = 8.6

Hz, H arom.), 8.12 (2H, d, J = 8.6 Hz, H arom.), 7.65 (1H, s, CH=N(O)), 2.89 (4H, s, CH_2-CO), 1.61 (9H, s, CH_3 from *tert*-butyl).

^{13}C NMR (62.86 MHz, $CDCl_3$): δ 169.3, 161.3 (CO), 136.8 (C^{IV} arom), 130.7 (CH arom.), 128.6 (C^{IV} arom), 128.6 (CH arom.), 125.5 (CH=N(O)), 72.2 (C^{IV}), 28.4 (CH_2-CO), 25.7 (CH_3 from *tert*-butyl).

Synthesis of [5-tert-butoxycarbonylamino-5-(3,3,4,4,5,-5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl-carbamoyl)pentyl]carbamic acid benzyl ester **3**



2.26 g of azide $C_8F_{17}CH_2CH_2N_3$ (4.62 mmol - 1 equiv.) are dissolved in 20 ml of ether. The medium is brought to 0°C and 300 mg of palladium-on-charcoal (10% - 65 mg/mmol) are added fractionwise. After stirring for 6 hours in the hydrogenation bomb (pressure 8 bar), the medium is filtered through a layer of celite and the solvents are eliminated under reduced pressure. The amine $C_8F_{17}CH_2CH_2NH_2$ **2** is obtained without purification.

1.79 g of Boc-Lys(Z)OH (4.70 mmol - 1 equiv.), 1.07 g of DCC (5.18 mmol - 1.1 equiv.) and 0.64 g of HOBt (4.70 mmol - 1 equiv.) are dissolved in 20 ml of anhydrous dichloromethane. After stirring for 15 minutes, the amine **2** in solution in 10 ml of anhydrous dichloromethane is added to the mixture. The stirring is continued for 24 hours. The reaction crude is filtered through sintered glass (porosity 4) and the solvents were then eliminated under reduced pressure. After purification by flash chromatography on silica gel (eluant: 7:3 to 6:4 cyclohexane/ethyl acetate), the

fluorinated compound **3** (3.11 g - 3.70 mmol - 79%) is obtained in the form of a white powder.

Molar mass ($C_{29}H_{32}F_{17}N_3O_5$): 825.6 $g \cdot mol^{-1}$

Melting point: 86.5 - 88.3°C.

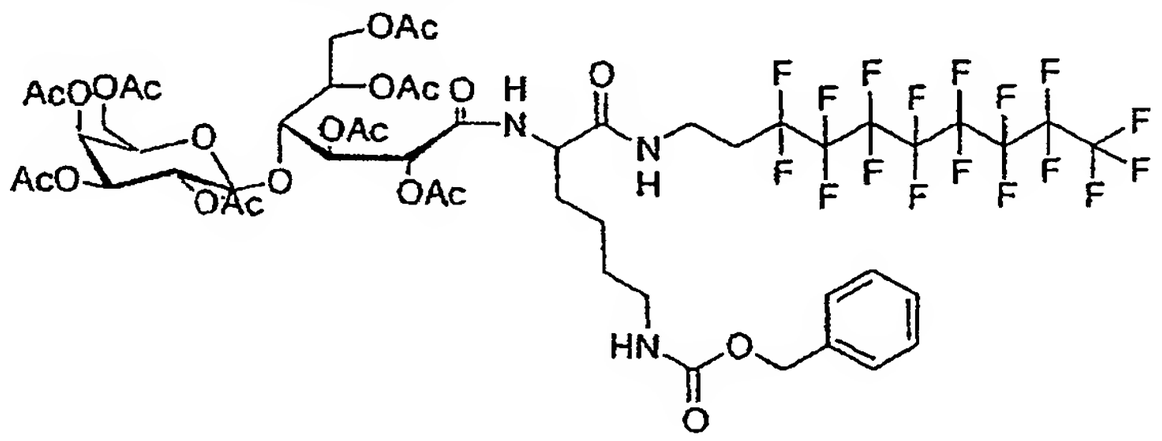
^1H NMR (250 MHz, $CDCl_3$): δ 7.36 (5H, s, CH arom.), 6.85 (1H, m, NH amide), 5.27 (1H, d, J = 6.65 Hz, NH urethane), 5.11 (2H, s, CH_2-O), 4.99 (1H, t, J = 5.8 Hz, NH urethane), 4.06 (1H, m, CH-CO), 3.58 (2H, dd, J = 6.5 Hz, CH_2-NH), 3.20 (2H, dd, J = 6.2 Hz, CH_2-NH), 2.35 (2H, m, CH_2-CF_2), 2.0 to 1.0 (15 H, m, CH_3 from Boc and CH_2 lys).

^{13}C NMR (62.86 MHz, $CDCl_3$): δ 172.5 (CO-NH), 156.7, 155.9 (O-CO-NH), 136.6 (C^{IV} arom.), 128.5, 128.1 (CH arom.), 80.4 (C^{IV}), 66.7 (CH_2-O-CO-NH), 54.4 (CH-CO), 40.2 (CH_2-NH), 31.9 (triplet, CH_2-NH), 31.3 (CH_2), 30.7 (CH_2-Rf), 29.5 (CH_2), 28.2 (CH_3 from *tert*-butyl), 22.4 (CH_2).

^{19}F NMR (235 MHz, DMSO-d6): δ -80.7 (CF_3, s), -113.9 ($\underline{CF_2}$-CF_3, s), -121.9 (3 CF_2, m), -122.7 (CF_2, s), -123.5 (CF_2, s), 126.0 (CF_2-CH_2, s).

$[\alpha]_D$ = -8.2 (c, l, $CHCl_3$).

Synthesis of LactoLys(Z)C_8F_{17}(OAc)$_8$ **5**



2.03 g of compound **3** (2.45 mmol - 1 equiv.) are dissolved in 20 ml of anhydrous dichloromethane. The medium is brought to 0°C and 40 ml of an 8.5:1.5 CH_2Cl_2/TFA mixture are added dropwise while keeping the temperature at °C throughout the addition. After stirring for 4 hours, the solvents are eliminated under reduced pressure. The crude is taken up in ether and then evaporated in order to eliminate the residual

traces of TFA by co-evaporation. The operation is repeated several times and produces the free amine **4**.

In parallel, 1.15 g of lactobionic acid (3.19 mmol - 1.3 equiv.) are suspended in 40 ml of a 1:1 methoxyethanol/toluene mixture acidified with 3 drops of TFA.

After evaporation at 45°C under reduced pressure, the medium is taken up in 30 ml of a 2:1 methoxyethanol/-toluene mixture and then evaporated to dryness. The latter operation is repeated twice so as to produce lactobionolactone.

The lactobionolactone and the amine **4** are dissolved, under an argon atmosphere, in 40 ml of methanol. The pH of the solution is brought to 9 by adding TEA, and the medium is then brought to reflux for 24 hours. After elimination of the methanol under reduced pressure, 40 ml of a 1:1 acetic anhydride/pyridine mixture are added, under cold conditions, to the crude. The stirring is maintained for 18 hours and the reaction mixture is then poured onto 150 ml of 1N HCl. The aqueous phase is extracted with 3 times 50 ml of dichloromethane. The organic phase is respectively washed with twice 60 ml of 1N HCl and then with 60 ml of brine and, finally, dried over Na_2SO_4. The solvents are eliminated under reduced pressure and the crude is purified by flash chromatography on silica gel (eluant: 6:4 to 7:3 ethyl acetate/cyclohexane) so as to produce the compound **5** (2.23 g - 1.59 mmol - 65%) in the form of a white powder.

Molar mass ($C_{52}H_{60}F_{17}N_3O_{22}$): 1402.0 $g \cdot mol^{-1}$

Melting point: 65°C (beginning of decomposition).

^1H NMR (250 MHz, DMSO-d6): δ 8.07 (2H, m, NH), 7.34 (5H, s, CH arom.), 7.01 (1H, m, NH), 5.47 (1H, m, H from the sugar), 5.30 to 5.10 (2H, m, H from the

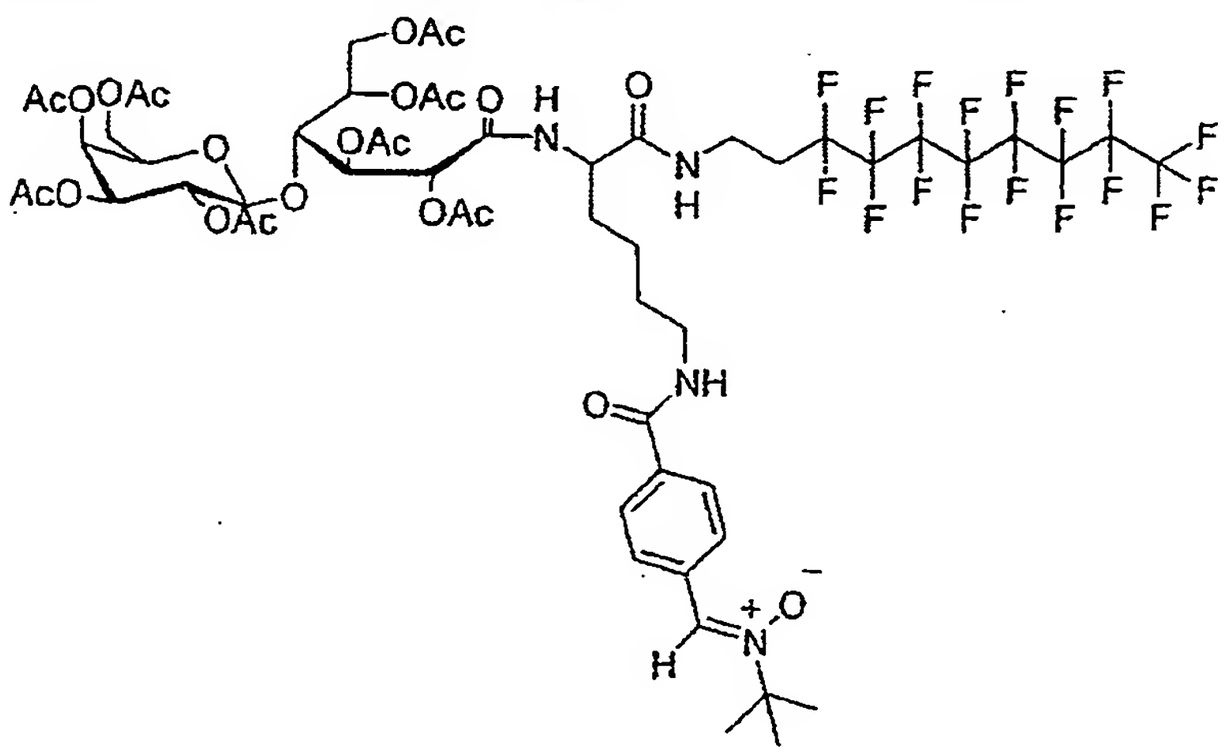
sugar), 5.02 to 4.79 (5H, m, CH_2-O and H from the sugar), 4.50 to 3.90 (8H, m, H from the sugar and CH from lysine), 3.38 (2H, m, CH_2-NH), 2.97 (2H, m, CH_2-NH), 2.30 (2H, m, CH_2-CF_2), 2.14, 2.09, 2.04, 2.01, 1.96, 1.92 (24H, 6s, CH_3 from acetyls), 1.65 to 1.10 (6H, m, CH_2 from lysine).

^{13}C NMR (62.86 MHz, $CDCl_3$): δ 171.3 (CO-NH), 170.5, 170.5, 170.1, 170.0, 170.0, 169.7, 169.2 (7s, CO-O), 167.9 (CO-NH), 156.7 (O-CO-NH), 136.7 (C^{IV} arom.), 128.4, 128.0, 127.9, (CH arom.), 101.6 (CH-1'), 77.9 (CH-4), 72.7 (CH-2), 71.1, 70.9 (CH-5' and CH-3'), 70.0 (CH-5), 69.4 (CH-3), 69.0 (CH-2'), 66.9 (CH-4'), 66.5 (CH_2-O), 61.6, 61.0 (CH_2-6 and CH_2-6'), 52.6 (CH-CO), 40.4 (CH_2-NH), 31.9 (triplet, CH_2-NH), 31.2 (CH_2-), 30.5 (triplet, CH_2-Rf), 29.1 (CH_2-), 22.2 (CH_2-), 20.6, 20.5, 20.4, 20.3 (5s, CH_3 from acetyls).

^{19}F NMR (235 MHz, DMSO-d6): δ -80.2 (CF_3, s), -113.0 ($C\underline{F_2}$-CF_3, s), -121.4 (3 CF_2, s), -122.2 (CF_2, s), -123.0 (CF_2, s), -125.4 (CF_2-CH_2, s).

$[\alpha]_D$ = +2.9 (c, 1, $CHCl_3$).

Synthesis of LactoLys(PBN)C_8F_{17}(OAc)$_8$ **7**



0.400 g of compound **5** (0.28 mmol - 1 equiv.) is dissolved in 10 ml of dioxane. The medium is brought to 0°C and 0.190 g of palladium-on-charcoal (10% - 65 mg/mmol) are added fractionwise. After stirring for 20 hours in the hydrogenation bomb (pressure 8 bar), the medium is filtered through a layer of celite and

the solvents are eliminated under reduced pressure. The amine **6** is obtained in the form of a white powder without purification.

The amine **6** is dissolved, under a stream of argon, in 5 ml of anhydrous dichloromethane. 0.090 g of active ester **X** (0.28 mmol - 1 equiv.) is added to the medium and the pH is brought to 9 by adding DIEA.

The stirring is continued under an argon atmosphere for 24 hours. The solvents are evaporated off under reduced pressure and the crude is purified by flash chromatography on silica gel (eluant: ethyl acetate). A further purification by size exclusion chromatography on Sephadex LH-20 resin (eluant: 1:1 dichloromethane/ethanol) makes it possible to obtain the nitrone **7** (0.230 g - 0.156 mmol - 54%) in the form of a white powder.

Molar mass ($C_{56}H_{67}F_{17}N_4O_{22}$): 1471.1 $g \cdot mol^{-1}$

Melting point: 75°C (beginning of decomposition).

^1H NMR (250 MHz, $CDCl_3$): δ 8.36 (2H, d, J = 8.3 Hz, H arom.), 7.92 (2H, d, J = 8.4 Hz, H arom.), 7.68 (1H, s, CH=N(O)), 6.94 (3H, m, NH), 5.51 (1H, dd, J = 2 Hz and J = 4.4 Hz, H-4'), 5.32 (2H, m, H-2 and H-3), 5.17 to 4.95 (3H, m, H-2', H-5 and H-3'), 4.62 (1H, d, J = 7.7 Hz, H-1'), 4.50 (1H, dd, J = 2.7 Hz and J = 12.5 Hz, H from the sugar), 4.33 (1H, m, CH from the lysine), 4.18 (1H, dd, J = 1.6 Hz and J = 6.3 Hz, H from the sugar), 4.12 to 3.87 (4H, m, H from the sugar and H-5'), 3.50 (2H, m, CH_2-NH), 3.39 (2H, m, CH_2-NH), 2.35 (2H, m, CH_2-CF_2), 2.15, 2.09, 2.08, 2.04, 2.02, 2.00, 1.96 (24H, 7s, CH_3 from acetyls), 1.60 (11H, m, CH_3 from *tert*-butyl and CH_2 Lys), 1.85 (2H, m, CH_2 Lys), 1.35 (2H, m, CH_2 Lys).

^{13}C NMR (62.86 MHz, $CDCl_3$): δ 171.5 (CO-NH), 170.6, 170.5, 170.3, 170.3, 170.0, 169.7, 169.32 (7s, CO-O), 168.2 (CO-NH), 167.1 (CO-NH), 135.2 (C^{IV} arom.),

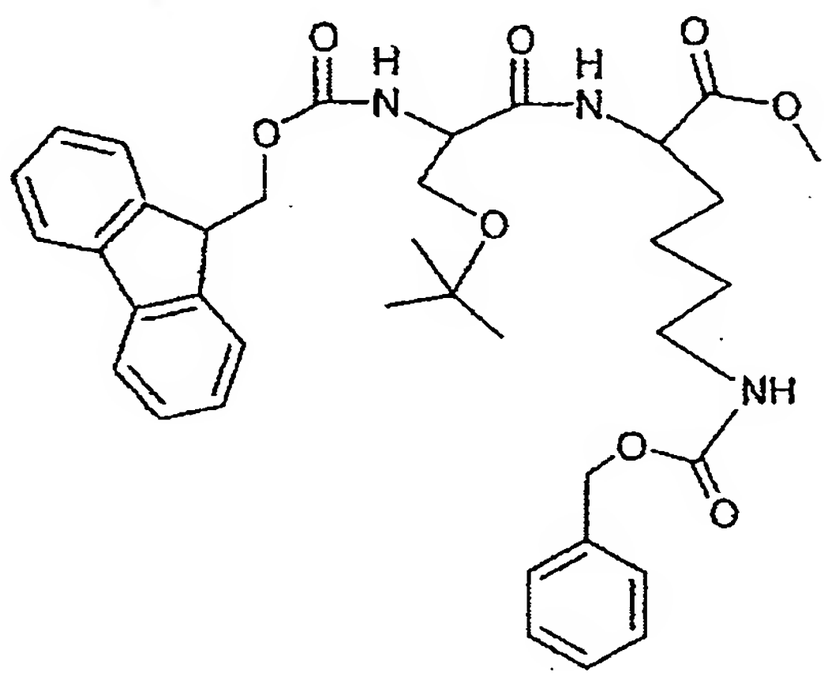
133.7 (C^{IV} arom.), 129.3 (CH=N(O)), 128.6, 127.3 (CH arom.), 101.7 (CH-1'), 78.5 (CH-4), 72.9 (CH-2), 71.4 (C^{IV}), 71.0, 70.9 (CH-5' and CH-3'), 68.9 (CH-5), 69.3 (CH-3), 69.0 (CH-2'), 66.9 (CH-4'), 61.6, 61.1 (CH_2-6 and CH_2-6'), 52.7 (CH-CO), 39.4 (CH_2-NH), 31.9 (m, CH_2-NH), 31.1 (CH_2-), 30.5 (triplet, CH_2-Rf), 28.7 (CH_2-), 28.2 (CH_3 from *tert*-butyl), 22.2 (CH_2-), 20.7, 20.7, 20.7, 20.6, 20.5, 20.5, 20.4, (7s, CH_3 from acetyls).

^{19}F NMR (235 MHz, $CDCl_3$): δ -80.7 (s, CF_3), -114.2 (s, $\underline{CF_2}$-CF_3), -121.9 (s, 3 CF_2), -122.7 (s, CF_2), -123.5 (s, CF_2), -126.1 (s, CF_2-CH_2).

$[\alpha]_D$ = +1.6 (c, 1, $CHCl_3$).

3/ Example 3:

A- Synthesis of compound B



1

0.99 g (3×10^{-3} mol) of $Cl^{-+}H_3N$ Lys(Z) OMe are dissolved in 10 ml of dichloromethane, in a 100 ml round-bottomed flask. The pH is brought to 8 with TEA. 1.15 g (3×10^{-3} mol, 1 eq.) of Fmoc Ser(OtBu) OH are then added, along with 1.72 g (3.9×10^{-3} mol, 1.3 eq.) of BOP.

The pH is maintained at 8 throughout the reaction. The medium is kept stirring at ambient temperature for 24 hours. Once the reaction is complete (TLC), the organic phase is washed with 1N HCl and then with $NaHCO_3$ in order to reestablish a pH of 7. The organic phase is dried over Na_2SO_4, filtered, and then evaporated under

reduced pressure. Crystallization can be carried out from a dichloromethane/Et_2O mixture. 1.93 g of the **compound 1** are obtained in the form of a white powder.

Yield: 98%.

Rf = 0.58 in 6 ethyl acetate/4 cyclohexane.

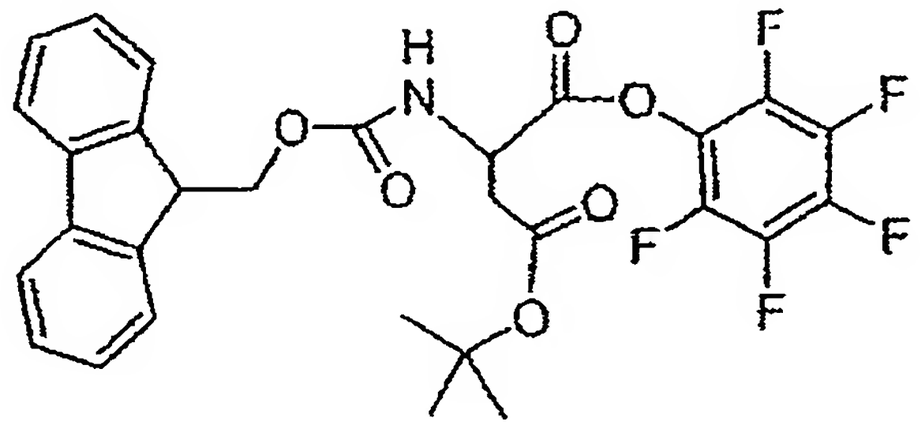
Degradation temperature: 80.6°C. $[\alpha]^{D}_{20}$ = +17.9 (c, 1, CH_2Cl_2).

^1H NMR in $CDCl_3$:

δ = 7.75 (2H, d, ar Fmoc); 7.60 (2H, d, ar Fmoc); 7.43-7.21 (5H, m, ar Fmoc, N**H** Lys); 7.31 (5H, s, ar Z Lys); 5.78 (1H, d, N**H** Ser); 5.08 (2H, s, C**H$_2$**, Z Lys); 4.84 (1H, t, N**H** Z Lys); 4.60 (1H, m, C**H** Lys); 4.39 (2H, d, C**H$_2$** Fmoc); 4.24 (2H, m, C**H** Ser, C**H** Fmoc); 3.81 (1H, dd, C**H$_2$** Ser); 3.73 (3H, s, OC**H$_3$** Lys); 3.39 (1H, dd, C**H$_2$** Ser); 3.17 (2H, C**H$_2$**-NH Lys); 1.85-1.29 (6H, m, CH-C**H$_2$**-C**H$_2$**-C**H$_2$** Lys); 1.22 (9H, s, tBu Ser).

^{13}C NMR in $CDCl_3$:

δ = 172.94 (**C**O Lys); 170.88 (**C**O Ser); 157.08; 156.67 (2 O-**C**O-NH Fmoc and Z); 144.52; 144.36; 141.93; 137.23; 129.13; 128.71; 128.35; 127.71; 125.76; 120.62 (**C** ar Fmoc and Z); 74.96 (**C**$(CH_3)_3$ Ser); 67.80; 67.24 (**C**H_2 Fmoc and **C**H_2 Z); 62.39 (C*-**C**H_2 Ser); 54.93 (**C***-CH_2 Ser); 52.99; 52.74 (O**C**H_3 Lys and **C***-CH_2 Lys); 47.76 (**C**H Fmoc); 41.27 (**C**H_2-NH-CO-O Lys); 32.74 (**C**H_2-CH_2-NH-CO-O Lys); 30.02 (C*-**C**H_2 Lys); 27.99 (C(**C**$H_3)_3$ Ser); 22.92 (C*-CH_2-**C**H_2 Lys).



2

0.68 g (1.53×10^{-3} mol) of Fmoc Asp(OtBu)OH is dissolved, in 10 ml of dichloromethane, along with 0.34 g (1.83×10^{-3} mol, 1.2 eq) of pentafluorophenol and 0.38 g

(1.83×10^{-3} mol, 1.2 eq) of DCC, in a 50 ml round-bottomed flask. The reaction is left stirring at ambient temperature for 15 hours. After filtration, the medium is concentrated under reduced pressure. The residual oil is chromatographed on silica gel (eluant: 2/8: ethyl acetate/cyclohexane). Crystallization is carried out from ethyl acetate/hexane. 760 mg of **2** are obtained in the form of a white powder.

Yield: 86.2%.

Melting point: 94.6-95.8°C.

$[\alpha]^{D}_{20}$ = -2.3 (c, 1, CH_2Cl_2).

^{1}H NMR in $CDCl_3$:

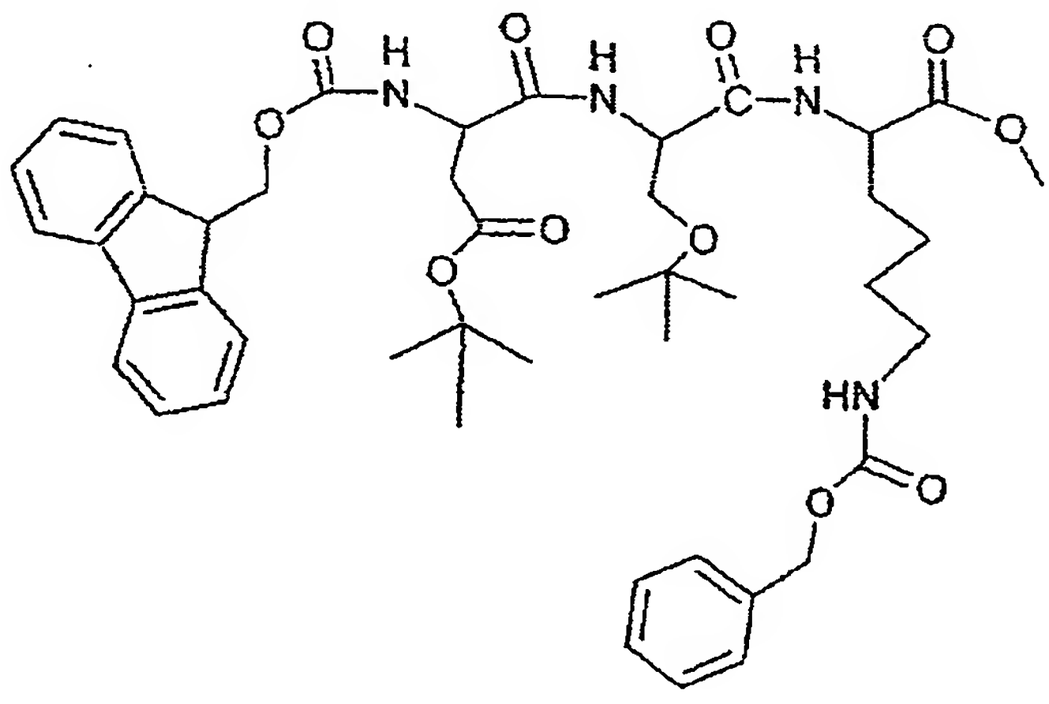
δ = 7.77 (2H, d, ar Fmoc); 7.61 (2H, d, ar Fmoc); 7.43-7.28 (4H, m, ar Fmoc); 5.98 (1H, d, NH); 4.98 (1H, m, CH Asp); 4.49-4.23 (3H, m, CH_2 Fmoc, CH Fmoc); 3.15 (1H, dd, $C\underline{H_2}$ Asp); 2.90 (1H, dd, $C\underline{H_2}$ Asp); 1.48 (9H, s, tBu Asp).

^{13}C NMR in $CDCl_3$:

δ = 170.35; 168.09 (2 $\underline{C}O$-O); 156.55 (O-$\underline{C}O$-NH Fmoc); 144.43; 144.26; 142.01; 128.47; 127.78; 125.79; 120.71 ($\underline{C}$ ar Fmoc); 143.82-136.34 ($\underline{C}F$); 83.39 ($\underline{C}(CH_3)_3$ Asp); 68.21 ($\underline{C}H_2$ Fmoc); 50.97 ($\underline{C}$*-CH_2 Asp); 47.76 ($\underline{C}H$ Fmoc); 38.31 (C*-$\underline{C}H_2$ Asp); 28.68 ($C(\underline{C}H_3)_3$ Asp).

^{19}F NMR in $CDCl_3$:

δ = -152.22 (2F); -157.36 (1F); -162.10 (2F)



3

Before the coupling between the dipeptide and the

activated amino acid, a step consisting of deprotection of the compound **2** is necessary. For this, 1.3 g (1.97 mmol) of the dipeptide **1** are dissolved in 15 ml of a 10% v/v piperidine/dichloromethane mixture. The medium is left to stir for two hours at ambient temperature and is then washed with a separating funnel, with a 1N HCl solution. The organic phase is then washed with a saturated sodium hydrogen carbonate solution. The organic phases are subsequently dried over sodium sulfate and then concentrated under reduced pressure. The coupling can then be carried out.

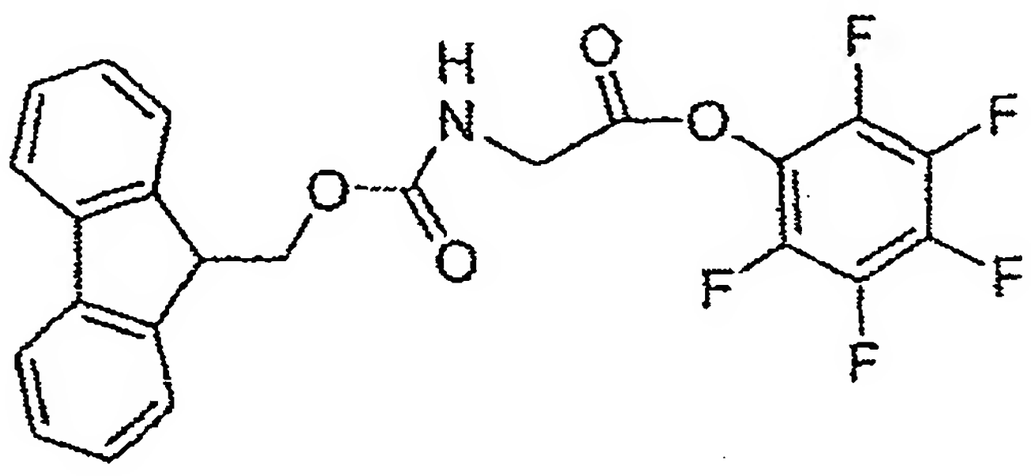
The dipeptide deprotected above is dissolved in 20 ml of dichloromethane, in a 100 ml single-necked round-bottomed flask, in the presence of 1.138 g (1.97 mmol, 1 eq) of compound **2** added to the round-bottomed flask. The reaction takes place at ambient temperature under a stream of nitrogen, in the dark, at a pH of 8 fixed with DIEA. After 15 hours, the reaction is complete (TLC). The reaction medium is concentrated and the residue is chromatographed on silica gel in a 5/5 ethyl acetate/cyclohexane eluant mixture. After evaporation of the solvent, 970 mg of compound **3** are obtained in the form of a translucent gel.

Yield: 59.2%.

^{1}H NMR in $CDCl_3$: δ = 7.78 (2H, d, ar Fmoc); 7.61 (2H, d, ar Fmoc); 7.46-7.23 (6H, m, ar Fmoc, N**H** Lys, N**H** Ser); 7.36 (5H, s, H ar Z Lys); 5.93 (1H, d, N**H** Asp); 5.11 (2H, s, C**H$_2$** Z Lys); 4.91 (1H, t, N**H** Z Lys); 4.57 (2H, m, C**H** Asp, C**H** Lys); 4.48-4.42 (3H, m, C**H** Ser, C**H$_2$** Fmoc); 4.26 (1H, t, C**H** Fmoc); 3.85 (1H, dd, C**H$_2$** Ser); 3.74 (3H, s, OC**H$_3$** Lys); 3.41 (1H, dd, C**H$_2$** Ser); 3.17 (2H, C**H$_2$**-NH Lys); 2.88 (1H, dd, C**H$_2$** Asp); 2.72 (1H, dd, CH_2 Asp); 1.48 (9H, s, tBu Asp); 1.84-1.28 (6H, m, CH-C**H$_2$**-C**H$_2$**-C**H$_2$** Lys); 1.22 (9H, s, tBu Ser).

^{13}C NMR in $CDCl_3$: δ = 172.96; 171.74; 171.14; 170.52 (**C**O Lys, **C**O Ser, 2 **C**O Asp); 157.06; 156.65 (2 O-**C**O-NH Fmoc and Z); 144.32; 141.94; 137.28; 129.14;

128.71; 128.41; 127.76; 125.72; 120.65 (**C** ar Fmoc and Z); 82.59 (**C**$(CH_3)_3$ Asp); 74.80 (**C**$(CH_3)_3$ Ser); 68.04; 67.88 (**C**H_2 Fmoc and **C**H_2 Z); 61.61 (C*-**C**H_2 Ser); 53.99 (**C***-CH_2 Ser); 52.95; 52.70 (O**C**H_3 Lys and **C***-CH_2 Lys); 52.04 (**C***-CH_2 Asp); 47.74 (**C**H Fmoc); 41.32 (**C**H_2-NH-CO-O Lys); 38.25 (C*-**C**H_2 Asp); 32.55 (**C**H_2-CH_2-NH-CO-O Lys); 29.93 (C*-**C**H_2 Lys); 28.67 (C(**C**$H_3)_3$ Asp); 27.97 (C(**C**$H_3)_3$ Ser); 22.99 (C*-CH_2-**C**H_2 Lys).



4

1 g (3.36×10^{-3} mol) of Fmoc Gly OH, 0.681 g of pentafluorophenol (3.7×10^{-3} mol, 1.1 eq) and 0.764 g of DCC (3.7×10^{-3} mol, 1.1 eq) are dissolved in 10 ml of dichloromethane. The medium is left at ambient temperature for 24 hours. The reaction medium is subsequently filtered, and the filtrate is then concentrated by evaporation under reduced pressure. The residue is subjected to flash chromatography on silica gel with a 5/5 ethyl acetate/cyclohexane eluant mixture. Crystallization is carried out from an ethyl acetate/cyclohexane mixture. 955 mg of **4** are obtained in the form of a white powder. Yield: 61.3%.

Melting point: 156.3-157.4.

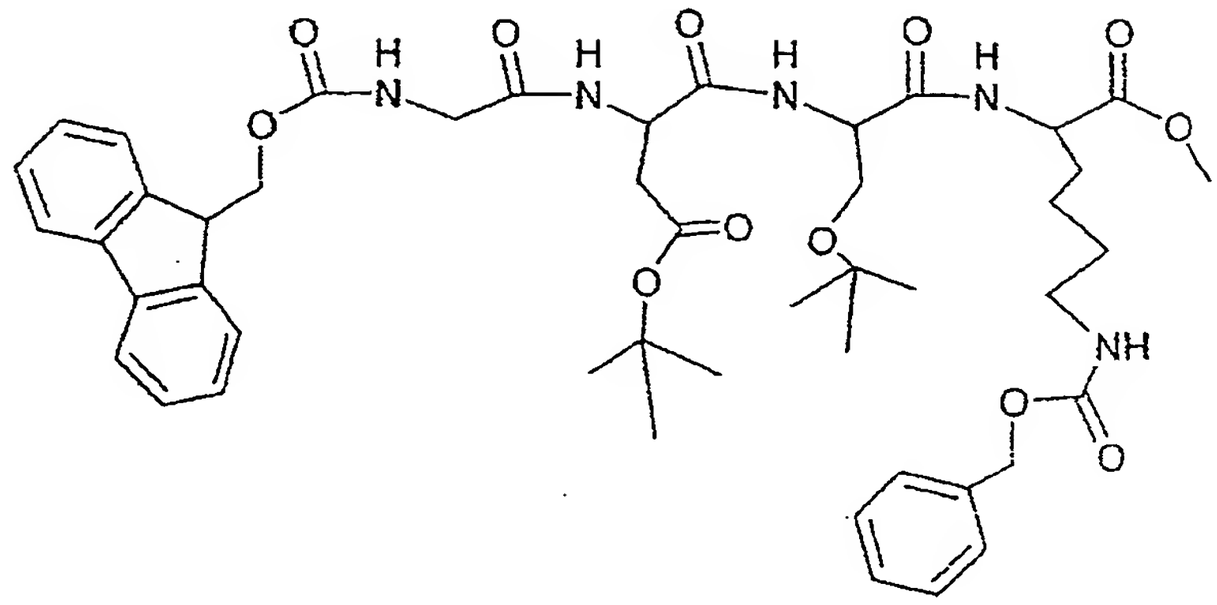
$[\alpha]^{D}_{20} = -1.3$ (c, 1, CH_2Cl_2).

^{1}H NMR in $CDCl_3$: δ = 7.82 (2H, d, ar Fmoc); 7.65 (2H, d, ar Fmoc); 7.47-7.25 (4H, m, ar Fmoc); 5.40 (1H, t, NH); 4.54-4.42 (5H, m, CH_2 Fmoc, CH Fmoc, CH_2 Gly).

^{13}C NMR in $CDCl_3$: δ = 167.14 (**C**O-O Gly); 156.84 (NH-**C**O-O); 144.31; 142.00; 128.46; 127.77; 125.66;

120.70 (**C** ar Fmoc); 68.14; (**C**H_2 Fmoc); 47.73 (**C**H Fmoc); 42.87 (**C**H_2 Gly).

^{19}F NMR in $CDCl_3$: δ = -152.44 (2F); -157.25 (1F); -161.96 (2F).



5

This synthesis is preceded by deprotection of the tripeptide according to the same protocol as for the synthesis of this tripeptide (compound **3**). 500 mg (6×10^{-4} mol) of **3** are deprotected. Once deprotected, this peptide is dissolved in 10 ml of dichloromethane in a 100 ml round-bottomed flask. 278 mg (6×10^{-4} mol, 1 eq) of compound **4** are added to the reaction medium. The reaction is carried out at ambient temperature under a stream of nitrogen, and is maintained at pH 8 with DIEA. After 16 hours, the reaction is complete (TLC). After evaporation of the medium under reduced pressure, the residual oil is chromatographed on silica gel with a 6/3 ethyl acetate/cyclohexane eluant mixture. From an ethyl acetate/cyclohexane mixture, 426 mg of **5** are obtained in the from of a white powder. Yield: 80%.

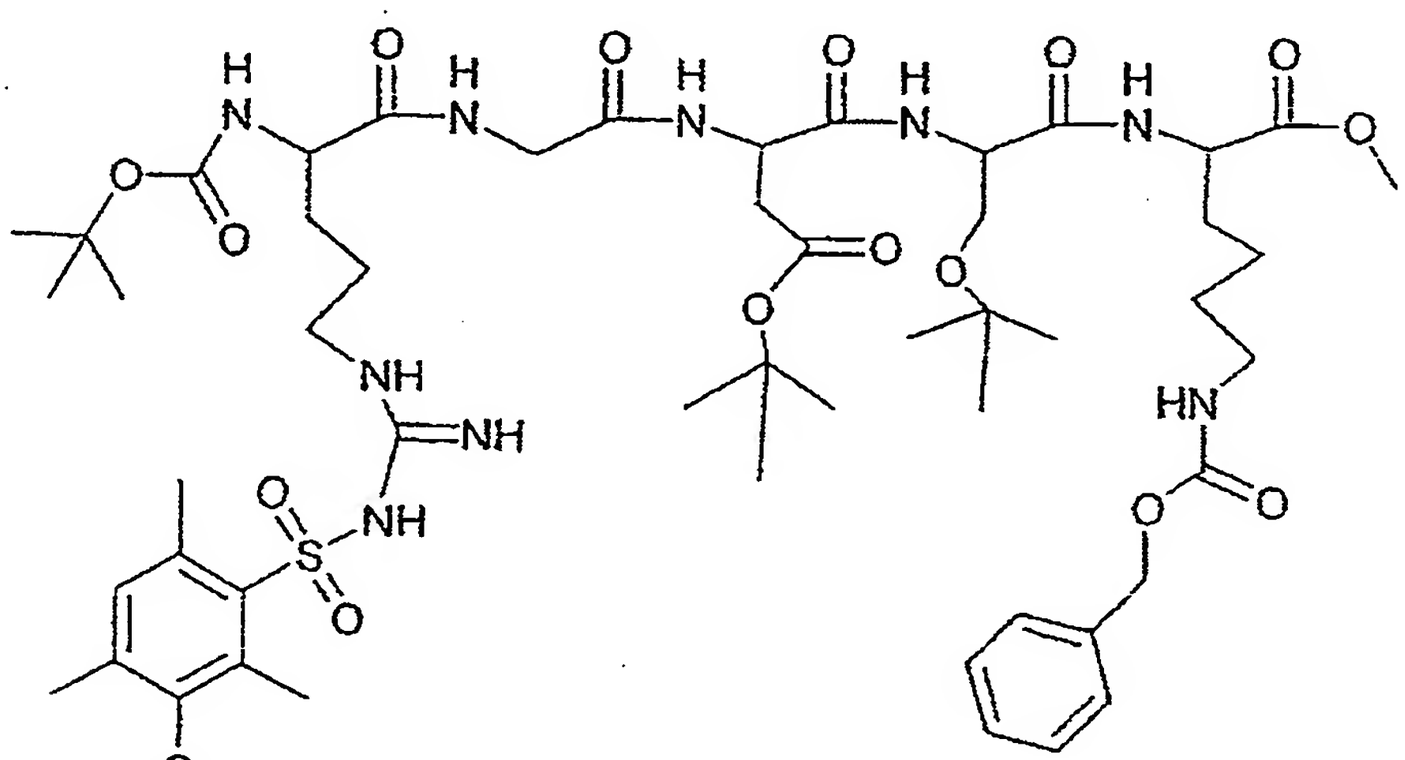
Degradation temperature: 63.7°C.

$[\alpha]^{D}_{20}$ = +4.6 (c, 1, CH_2Cl_2).

^1H NMR in $CDCl_3$: δ = 7.76 (2H, d, ar Fmoc); 7.60 (2H, d, ar Fmoc); 7.45-7.22 (7H, m, ar Fmoc, NH Lys, NH Ser, NH Asp,); 7.34 (5H, s, ar Z Lys); 5.83 (1H, d, NH Gly); 5.14 (1H, t, N**H** Z Lys) 5.07 (2H, s, CH_2 Z Lys); 4.82 (1H, m, CH Asp); 4.57 (1H, m, CH Lys); 4.45-4.35 (3H, m, CH Ser, CH_2 Fmoc); 4.20 (1H, t, CH Fmoc); 3.89

(2H, m, $C\underline{H_2}$ Gly); 3.75 (1H, dd, CH_2 Ser); 3.68 (3H, s, OCH_3 Lys); 3.39 (1H, dd, CH_2 Ser); 3.15 (2H, CH2-NH Lys); 2.88 (1H, dd, $C\underline{H_2}$ Asp); 2.68 (1H, dd, $C\underline{H_2}$ Asp); 1.41 (9H, s, tBu Asp); 1.91-1.29 (4H, m, CH-$C\underline{H_2}$-$C\underline{H_2}$ Lys); 1.22 (9H, s, tBu Ser).

^{13}C NMR in $CDCl_3$: δ = 173.00; 171.90; 170.75; 170.49; 169.76 (**C**O Lys, **C**O Ser, 2 **C**O Asp, **C**O Gly); 157.31; 157.13 (2 O-**C**O-NH Fmoc and Z); 144.45; 141.98; 137.27; 129.19; 128.78; 128.42; 127.78; 125.77; 120.68 (**C** ar Fmoc and Z); 82.78 (**C**$(CH_3)_3$ Asp); 74.78 (**C**$(CH_3)_3$ Ser); 68.05; 67.30 (**C**H_2 Fmoc and **C**H_2 Z); 61.60 (C*-**C**H_2 Ser); 54.19 (**C***-CH_2 Ser); 53.01; 52.72 (OCH_3 Lys and **C***-CH_2 Lys); 50.28 (**C***-CH_2 Asp); 47.76 (**C**H Fmoc); 45.26 (**C**H_2 Gly); 41.38 (**C**H_2-NH-CO-O Lys); 37.68 (C*-**C**H_2 Asp); 32.58 (**C**H_2-CH_2-NH-CO-O Lys); 29.99 (C*-**C**H_2 Lys); 28.67 ($C(\underline{C}H_3)_3$ Asp); 28.00 ($C(\underline{C}H_3)_3$ Ser); 23.03 (C*-CH_2-**C**H_2-Lys).



6

The deprotection of the pentapeptide **5** is carried out under the same conditions as in the synthesis of compound **1**. 400 mg (4.5×10^{-4} mol) of the tetrapeptide are deprotected. Once the deprotection is complete, this tetrapeptide is dissolved in 15 ml of dichloromethane in a 50 ml single-necked round-bottomed flask. 219 mg (4.5×10^{-4} mol, 1 eq) of Boc Arg(Mtr) OH and 188 mg (5.85×10^{-4} mol, 1.3 eq) of TBTU are added. The reaction is carried out at ambient temperature under a stream of nitrogen, in the dark, and is

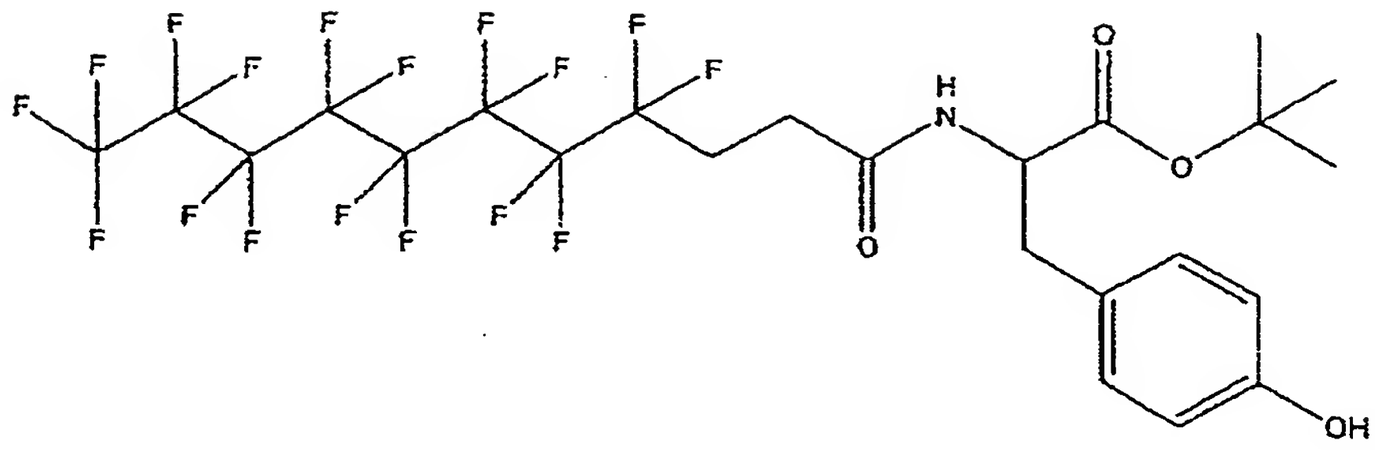
maintained at pH 8 with DIEA. After 16 hours, the reaction is complete (TLC). After evaporation under reduced pressure, the residual oil is chromatographed on silica gel using a 9/1 ethyl acetate/cyclohexane eluant. Crystallization is carried out from ethyl acetate/cyclohexane. 417 mg of **6** are obtained in the form of a white powder. Yield: 92.5%.

Degradation point: 80.2°C.

$[\alpha]^{D}_{20}$ = -2.9 (c, 1, CH_2Cl_2).

^1H NMR in $CDCl_3$: δ = 7.64 (1H, d, N**H** Asp); 7.53 (1H, d, N**H** Gly); 7.49-7.29 (7H, m, ar Z Lys, N**H** Lys, N**H** Ser); 6.54 (1H, s, **H** ar Mtr); 6.34-6.15 (3H, m, 3 N**H** guanidine Arg); 5.65 (1H, d, N**H** Boc Arg); 5.24 (1H, t, N**H** Z Lys); 5.10 (2H, s, C**H**$_2$ Z Lys); 4.77 (1H, m, C**H** Asp); 4.46 (2H, m, C**H** Lys, C**H** Ser); 4.22 (1H, m, C**H** Arg); 3.89-3.70 (3H, m, C**H**$_2$ Gly, 1H from C**H**$_2$ Ser); 3.84 (3H, s, OC**H**$_3$ Mtr Arg); 3.71 (3H, s, OC**H**$_3$ Lys); 3.50 (1H, dd, C**H**$_2$ Ser); 3.21 (2H, m, CH_2-C**H**$_2$-NH Arg); 3.17 (2H, C**H**$_2$-NH Lys); 2.90-2.64 (8H, m, 2 C**H**$_3$ Mtr Arg, C**H**$_2$ Asp); 2.15 (3H, s, C**H**$_3$ Mtr Arg); 1.84-1.19 (37H, m, tBu Asp, tBu Ser, tBu Boc Arg, CH-C**H**$_2$-C**H**$_2$ Lys, CH-C**H**$_2$-C**H**$_2$ Arg).

^{13}C NMR in $CDCl_3$: δ = 174.32; 174.13; 172.85; 171.48; 171.35; 170.92; 170.18 (**C**O Lys, **C**O Ser, 2 **C**O Asp, **C**O Gly, **C**O Arg, NH-**C**-NH Arg); 158.96; 157.20; 156.55 (2 O-**C**O-NH Boc and Z, **C**-OCH_3 Mtr Arg); 139.07; 137.25; 137.06; 134.19; 129.07; 128.87; 128.61; 125.30, 112.28 (**C** ar Mtr and Z); 82.37 (**C**$(CH_3)_3$ Asp); 80.53 (**C**$(CH_3)_3$ Arg); 74.63 (**C**$(CH_3)_3$ Ser); 67.09 (**C**H_2 Z); 60.96 (C*-**C**H_2 Ser); 55.99 (C-O**C**H_3 Mtr Arg); 54.57; 54.23 (**C***-CH_2 Ser, **C***-CH_2 Arg); 52.87; 52.74 (O**C**H_3 Lys and **C***-CH_2 Lys); 50.37 (**C***-CH_2 Asp); 43.92 (**C**H_2 Gly); 41.25; 40.75 (**C**H_2-NH-CO-O Lys, **C**H_2-NH-C-NH Arg); 37.59 (C*-**C**H_2 Asp); 32.27 (**C**H_2-CH_2-NH-CO-O Lys); 30.33; 29.83 (C*-**C**H_2 Lys, C*-**C**H_2 Arg); 28.94; 28.56; 27.86 (C(**C**$H_3)_3$ Asp, C(**C**$H_3)_3$ Ser, C(**C**$H_3)_3$ Arg); 25.93 (C*-CH_2-**C**H_2 Arg); 24.74 (C*-CH_2-**C**H_2 Lys); 23.06; 18.95; 12.52 (3 **C**H_3 Mtr Arg).



7

500 mg (1.01×10^{-3} mol) of $C_8F_{17}CH_2CH_2COOH$, 278 mg of H_2N TYR(OH) OtBu (1.01×10^{-3} mol, 1 eq) and 251 mg of DCC (1.2×10^{-3} mol, 1.2 eq) are dissolved in 10 ml of DMF, in a 50 ml round-bottomed flask. The reaction is carried out at ambient temperature for 24 hours under a stream of nitrogen, in the dark, and is maintained at pH 8 with DIEA. After evaporation of the DMF under reduced pressure, the residue is chromatographed on silica gel using a 2/8 ethyl acetate/cyclohexane eluant.

The product can be crystallized from ethyl acetate/n-heptane. 550 mg of **7** are obtained in the form of a white powder. Yield: 76%.

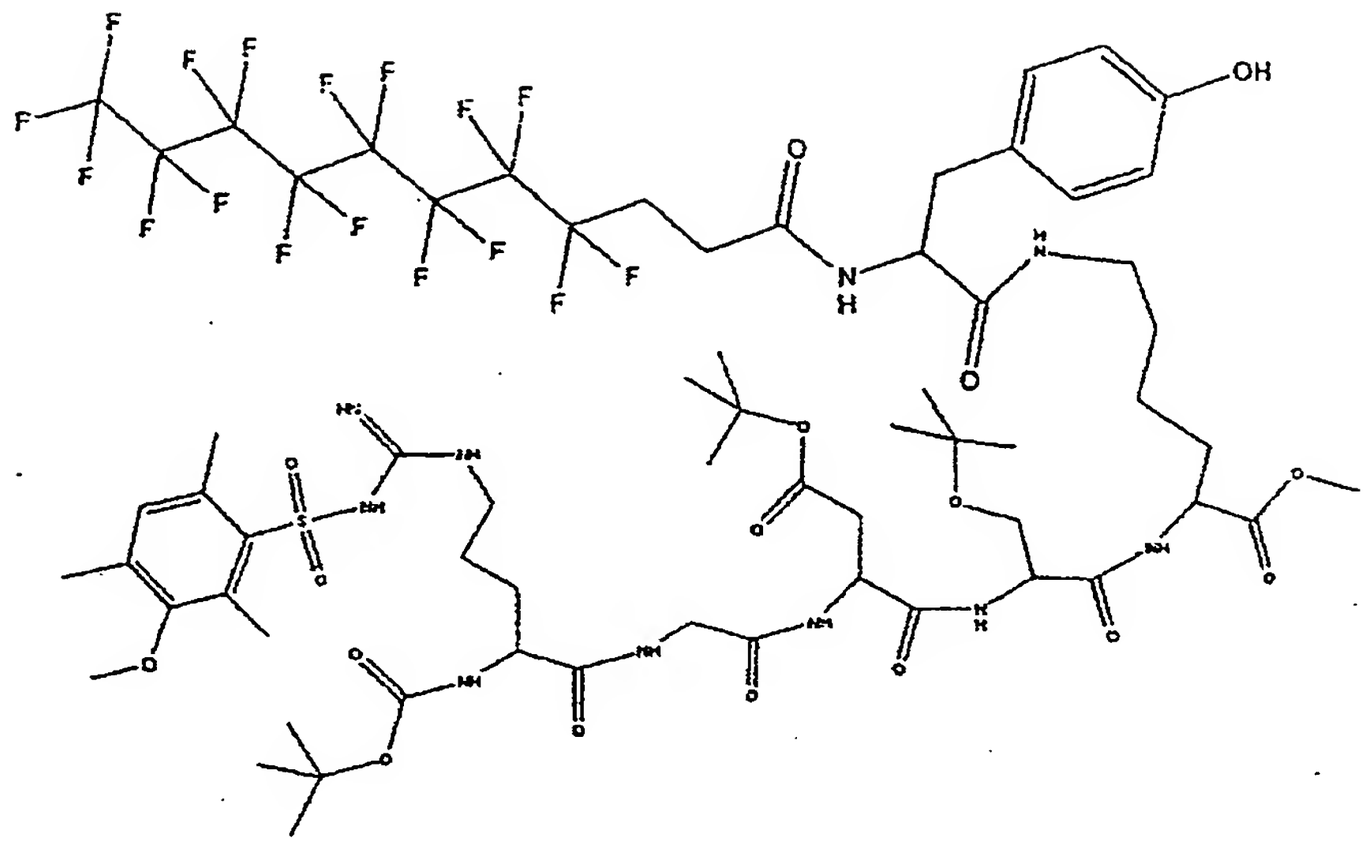
Melting point:

$[\alpha]^D_{20}$ = +30.0 (c, 1, CH_2Cl_2).

^1H NMR in $CDCl_3$: δ = 6.98 (2H, d, 2H ar Tyr); 6.72 (2H, d, 2H ar Tyr); 6.04 (1H, m, NH Tyr); 4.72 (1H, m, CH Tyr); 3.02 (2H, m, CF_2-CH_2-C**H$_2$**-CO); 2.47 (4H, m, CF_2-C**H$_2$**-CH_2-CO, C**H$_2$** Tyr); 1.44 (9H, s, tBu).

^{13}C NMR in $CDCl_3$: δ = 170.94 (**C**O-O); 169.52 (**C**O-NH); 155.13 (**C**-OH Tyr); 130.70 (**C** ar Tyr); 127.82 (C-**C**H ar Tyr); 115.49 (**C**H-C-OH ar Tyr); 122.5-106.3 (**C**F_3(**C**F_2)$_7$); 82.92 (**C**-(CH_3)$_3$)); 53.93 (NH-**C***-CO); 37.32 (C*-**C**H_2); 27.16 (**C**H_2-CF_2); 25.02 (**C**H_2-CO).

^{19}F NMR in $CDCl_3$: δ = -82.17; -115.50; -122.63; -123.49; -124.25; -127.06.



380 mg (5.34×10^{-4} mol) of compound **7** are dissolved, under cold conditions, in a TFA/CH_2Cl_2 (3/7) solution. After stirring for 2 hours, the deprotection is complete. The reaction medium is subsequently concentrated and then precipitated from ether several times. After evaporation, a white powder is obtained (compound **8**).

150 mg (1.32×10^{-4} mol) of compound **6** are dissolved in methanol. 8 mg of palladium-on-charcoal at 10% are added under cold conditions. The reaction medium is placed under 8 atmospheres of hydrogen. After 2 h 30 min, the reaction is complete. The mixture is filtered through celite 521 and the filtrate is concentrated under reduced pressure (compound **9**).

Compound **9** (1.32×10^{-4} mol) is dissolved in 10 ml of DMF, in a 50 ml round-bottomed flask. 95 mg of compound **8** (1.45×10^{-4} mol, 1.1 eq) and 76 mg of TBTU (1.72×10^{-4} mol, 1.3 eq) are added. The reaction is carried out at ambient temperature for 24 hours under a stream of nitrogen, in the dark, and is maintained at pH 8 with DIEA. The reaction medium is concentrated under reduced pressure. The residue is chromatographed on silica gel using an ethyl acetate eluant. 141 mg of product **10** are

obtained in the form of a white powder. Yield 65.3%.

Degradation temperature: 76.8°C.

$[\alpha]^{D}_{20}$ = -5.4 (c, 1, CH_3OH).

^1H NMR in CD_3OD: δ = 7.06 (2H, d, 2H ar Tyr); 6.74 (1H, s, **H** ar Mtr); 6.69 (2H, d, 2H ar Tyr); 4.80-4.53 (4H, m, C**H** Asp, C**H** Lys, C**H** Ser C**H** Tyr); 4.04 (1H, m, C**H** Arg); 3.88-3.85 (5H, m, C$\mathbf{H_2}$ Gly, OC$\mathbf{H_3}$ Mtr Arg); 3.72-3.63 (5H, m, OC$\mathbf{H_3}$ Lys, C$\mathbf{H_2}$ Ser); 3.22-2.31 (18H, m, CH_2-C$\mathbf{H_2}$-NH Arg, C$\mathbf{H_2}$-NH Lys, 2 C$\mathbf{H_3}$ Mtr Arg, C$\mathbf{H_2}$ Asp, CF_2-CH_2-C$\mathbf{H_2}$-CO, CF_2-C$\mathbf{H_2}$-CH_2-CO, C$\mathbf{H_2}$ Tyr); 2.15 (3H, s, C$\mathbf{H_3}$ Mtr Arg); 1.85-1.20 (37H, m, tBu Asp, tBu Ser, tBu Boc Arg, CH-C$\mathbf{H_2}$-C$\mathbf{H_2}$-C$\mathbf{H_2}$ Lys, CH-C$\mathbf{H_2}$-C$\mathbf{H_2}$ Arg).

^{13}C NMR in $CDCl_3$: δ = 179.69; 174.13-173.95; 172.32; 172.07; 171.29; 171.10; 170.77; 170.21 (**C**O Lys, **C**O Ser, 2 **C**O Asp, **C**O Gly, **C**O Arg, NH-**C**-NH Arg, **C**O-NH Tyr, **C**O-NH, $C_8F_{17}CH_2CH_2$**C**ONH); 158.49; 156.80; 155.94 (O-**C**O-NH Boc, **C**-OCH_3 Mtr Arg, **C**-OH ar Tyr); 138.10; 136.49; 133.45; 129.97; 127.54; 124.31; 114.81; 111.41 (**C** ar Mtr, **C** ar Tyr); 81.22; 79.47 (**C**$(CH_3)_3$ Asp, **C**$(CH_3)_3$ Arg); 73.42 (**C**$(CH_3)_3$ Ser); 61.12 (C*-**C**H_2 Ser); 54.61; 52.24; 51.31; 49.91 (C-O**C**H_3 Mtr Arg, **C***-CH_2 Ser, **C***-CH_2 Arg, NH-**C***-CO Tyr, O**C**H_3 Lys and **C***-CH_2 Lys, **C***-CH_2 Asp); 42.29; 40.08; 38.60; 37.15; 36.69 (**C**H_2 Gly, **C**H_2-NH-CO-O Lys, **C**H_2-NH-C-NH Arg, C*-**C**H_2 Asp, C*-**C**H_2 Tyr); 30.77; 28.92; 28.25; 27.40; 26.96; 26.34; 25.95 (**C**H_2-CH_2-NH-CO-O Lys, C*-**C**H_2 Lys, C*-**C**H_2 Arg, C(**C**$H_3)_3$ Asp, C(**C**$H_3)_3$ Ser, C(**C**$H_3)_3$ Arg, **C**H_2-CF_2, C*-CH_2-**C**H_2 Lys); 22.94; 22.53 (1 **C**H_3 Mtr Arg, CF_2-CH2-**C**H2-CO); 17.42; 10.71 (2 **C**H_3 Mtr Arg).

Compound **10**, subjected to the action of a solution of trifluoroacetic acid in CH_2Cl_2 in the presence of thio-anizole (3; 5; 2) for 24 h, produces compound **B**, which is isolated in pure form after precipitation by adding ether to the solution, and chromatography on a Sephadex G50 column (eluant: H_2O). After lyophilization, the product **B** is in the form of a white powder.

B- Biological assays

The cytotoxicity of this molecule was tested on B16 melanoma cells; no toxicity could be measured up to concentrations of greater than 100 µM. After labeling with iodine 125, this molecule, injected intravenously into a batch of mice carrying a melanoma, accumulates in the stroma of the tumor and then diffuses slowly within the tumor (cf Table 2).

Tissue	15 min	30 min	1 h
Tumor (stroma)	3.9 ± 0.8	3.8 ± 0.9	4.2 ± 0.6
Tumor (center)	1.7 ± 0.6	1.8 ± 0.3	2.4 ± 0.4
Blood	5.8 ± 0.8	4.5 ± 0.8	3.6 ± 0.7
Liver	4.8 ± 0.8	2.2 ± 0.6	2.4 ± 0.7
Kidney	8.9 ± 1.6	5.9 ± 0.6	3.5 ± 0.6
Thyroid	5.9 ± 1.2	10.2 ± 2.5	15.3 ± 5.3

Table 2: Radioactivity measured in mice carrying B16 melanoma after IV injection of molecule **B** (10 µCi/animal).

4/ Example 4:

Methyl *N*-(*t*-butoxycarbonyl)-*N*-$^{\gamma}$-(2,3,6-trimethyl-4-methoxybenzenesulfonyl)-L-arginylglycinyl-*O*-(*t*-butyl)-L-aspartyl-*O*-(*t*-butyl)-L-serinyl-*N*-$^{\varepsilon}$-((4,4,5,-5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-undecanoyl)-*N*-$^{\varepsilon}$-(4-(4-[bis(2-chloroethyl)amino]phenyl)-butyramido)-L-lysinyl-L-tyrosinamido)-L-lysinate

The synthesis of compound **D** is comparable to those described above for the above compounds and is summarized in Figures 2A and 2B.

Physicochemical characteristics of **D**

Rf: 0.49 in ethyl acetate/methanol: 98/2.

MM: 2051.87 $g \cdot mol^{-1}$.

Degradation temperature: 134°C.

$[\alpha]^{D}_{20}$ = -0.77 (c, 0.1, CH_3OH).

MS (FAB): *m/z* 2052 $[M+H^+]$, 2074 $[M+Na]^+$.

^{1}H NMR (CD_3OD):

δ 7.03 (4H, m, 2H arom. Tyr, 2H arom. Chloramb.); 6.67 (5H, m, 2H arom. Tyr, 2H arom. Chloramb., H arom Mtr); 4.81; 4.47; 4.20; 4.01 (6H, 3m, H_α Tyr, $2H_\alpha$ Lys, H_α Asp, H_α Ser, H_α Arg); 3.82 (3H, s, CH_3 ether Mtr Arg); 3.68 (3H, s, CH_3 methyl ester Lys); 3.83-3.60 (12H, m, $2H_\alpha$ Gly, $2H_\beta$ Ser, 8H Chloramb.); 3.14-2.75 (10H, m, $4H_\varepsilon$ Lys, $2H_\delta$ Arg, $2H_\beta$ Asp, $2H_\beta$ Tyr); 2.67-2.48 (8H, m, $2H_\alpha$ and $2H_\beta$ fluorinated chain, $2H_\alpha$ Chloramb., $2H_\delta$ Chloramb.); 2.67; 2.61 (6H, 2m, $2CH_3$ Mtr Arg); 2.13 (5H, m, $2H_\beta$ Chloramb., CH_3 Mtr Arg); 1.93-1.16 (16H, m, $4H_\beta$ Lys, $4H_\gamma$ Lys, $4H_\delta$ Lys, $2H_\beta$ Arg, $2H_\gamma$ Arg); 1.44; 1.42; 1.16 (27H, 3s, 9 CH_3 *tert*-butyl ester Asp, *tert*-butyl ether Ser, *tert*-butyl urethane Arg).

^{13}C NMR (CD_3OD):

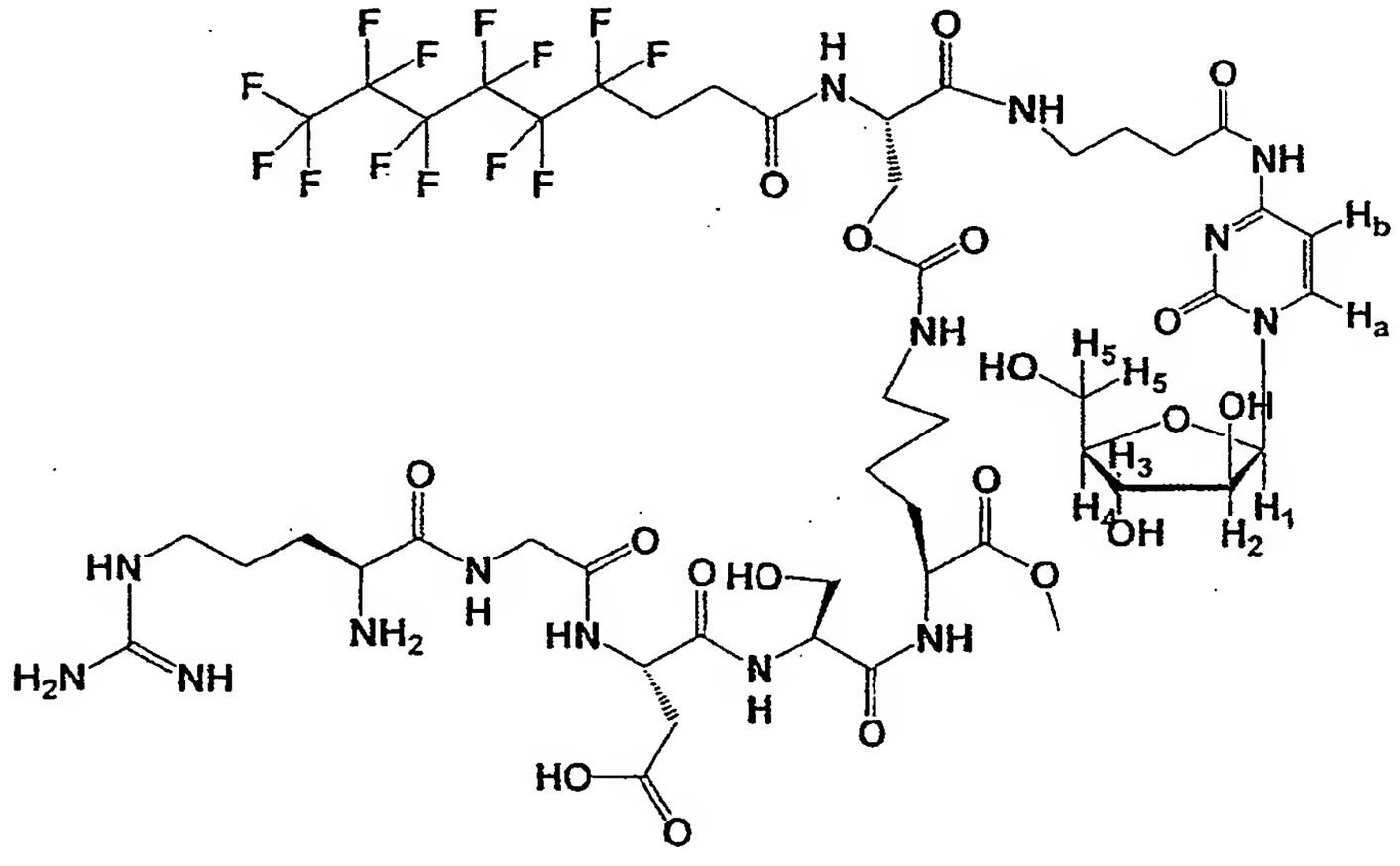
δ 174.67; 174.14; 172.57; 172.31; 171.80; 171.75; 171.26; 170.80; 170.17 (CO Tyr, 2CO Lys, CO Ser, 2 CO Asp, CO Gly, CO Arg, CO fluorinated chain, CO Chloramb.); 158.46; 156.80; 156.67; 155.93 (CO urethane Boc, C-OCH_3 arom. Mtr Arg, C-OH arom. Tyr, C guanidine Arg); 144.55 (C-N arom. Chloramb.); 138.10; 136.48; 133.42; 130.29; 129.97; 129.22; 127.51; 124.29; 114.83; 112.06; 111.38; 110.73 (C arom. Mtr, C arom. Tyr, C arom. Chloramb.); 81.17; 79.43 (C *tert*-butyl ester Asp, C *tert*-butyl urethane Arg); 73.40 (C *tert*-butyl ether Ser); 61.14 (C_β Ser); 54.96; 54.60; 54.53; 53.89; 53.14; 52.26; 51.31; 49.90 (CH_3 methyl ether Mtr Arg, C_α Ser, C_α Arg, C_α Tyr, CH_3 methyl ester Lys, $2C_\alpha$ Lys, C_α Asp, 2C-N Chloramb.); 42.30; 40.29; 38.58; 36.67; 36.52; 35.20 (C_α Gly, $2C_\varepsilon$ Lys, C_δ Arg, C_β Asp, C_β Tyr, 2C-Cl Chloramb.); 33.85; 31.02; 30.69; 28.91; 28.67; 28.15; 27.69; 27.41; 26.96; 26.36; 25.82; 25.46 ($2C_\delta$ Lys, $2C_\beta$ Lys, C_β Arg, C_γ Arg, 9 CH_3 *tert* butyl Asp, Ser, Arg, C_α-CF_2, $2C_\gamma$ Lys, C_α, C_γ Chloramb.); 23.00; 22.66 (C_β fluorinated chain, C_γ Chloramb.); 22.52; 17.47; 10.74 (3 CH_3 methyl Mtr Arg).

^{19}F NMR (CD_3OD):

δ -82.26 (3F); -115.39 (2F); -122.77 (6F); -123.62 (2F); -124.30 (2F); -127.17 (2F).

5/ Example 5:

Methyl L-arginyl-glycinyl-L-aspartyl-L-serinyl-*N*-$^{\varepsilon}$-(-O-(*N*-^{4}-(*N*-(4,4,5,5,6,6,7,7,8,8,9,9-tridecafluorononanoyl)-L-serinyl)-1-β-D-arabinofuranosylcytosine)-oxycarbonyl)-L-lysinate



Procedure:

The process for preparing F is identical to the previous processes; the synthesis is summarized in Figures 3A, 3B and 3C.

Physicochemical characteristics of **F**

MM: 1390.45 $g \cdot mol^{-1}$. MS (FAB): *m/z* 1391 $[M+H]^+$.

^{1}H NMR (DMSO, D_6):

δ 8.66 (1H, s, H acid Asp); 8.27-8.16; 7.94-7.84; 7.65; 7.21-6.92 (15H, 3m, H_a, NH GABA, 2NH Ser, 4H guanidine Arg, NH_2 Arg, NH Gly, NH Asp, 2NH Lys, NH Ara-C); 5.84 (1H, m, H_b); 5.31 (2H, m, $1H_\alpha$ Ser, H_1); 4.92 (1H, m, H_α Asp); 4.37-3.71 (17H, m, H_α Lys, H_α Ser, H_α Arg, $2H_\alpha$ Gly, $4H_\beta$ Ser, H_2, H_3, H_4, CH_3 methyl ester Lys, 2 H_5); 3.18-2.53 (14H, m, $2H_\varepsilon$ Lys, CH_2-NH GABA,

4OH, $2H_\beta$ Asp, 2 H_α and 2 H_β fluorinated chain); 2.23 (2H, m, CH_2-CO GABA); 1.46-1.03 (14H, m, $2H_\beta$, $2H_\gamma$, $2H_\delta$ Lys, $2H_\beta$, $2H_\gamma$, $2H_\delta$ Arg, CH_2 GABA).

^{13}C NMR (DMSO, D_6):

δ 173.9; 173.6; 173.4; 172.8; 171.1; 170.4; 170.1; 169.6; 169.1; 168.6 (CO Lys, 2 CO Ser, 2 CO Asp, CO Gly, CO Arg, CO fluorinated chain, CO GABA); 162.6; 157.4; 156.2; 155.0 (C guanidine Arg, CO urethane Ser, N=C-NH, N-CO-N); 147.2 (C_a); 94.8 (C_b); 87.5; 86.2 (C_1, C_4); 76.6; 75.0 (C_2, C_3); 65.4; 62.0; 61.5 (2 C_β Ser, C_5); 55.6; 53.0; 52.5; 52.3; 51.7; 50.1 (2 C_α Ser, C_α Arg, CH_3 methyl ester Lys, C_α Lys, C_α Asp); 42.5 (C_α Gly); 40.8; 38.5; 37.3; 34.8; 34.1; 30.9; 29.4; 29.0; 26.3; 24.8; 24.3; 23.0 (C_ε Lys, C_δ Arg, CH_2-NH GABA, C_β Asp, C_δ Lys, C_β Lys, CH_2-CO GABA, C_β Arg, C_α and C_β fluorinated chain, C_γ Arg, CH_2 GABA, C_γ Lys).

^{19}F NMR (DMSO, D_6):

δ -82.30 (3F); -115.51 (2F); -122.81 (6F); -123.82 (2F); -124.46 (2F); -127.21 (2F).